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Department of Agriculture.

REPORT

OF THE

Government

Veterinary Bacteriologist,

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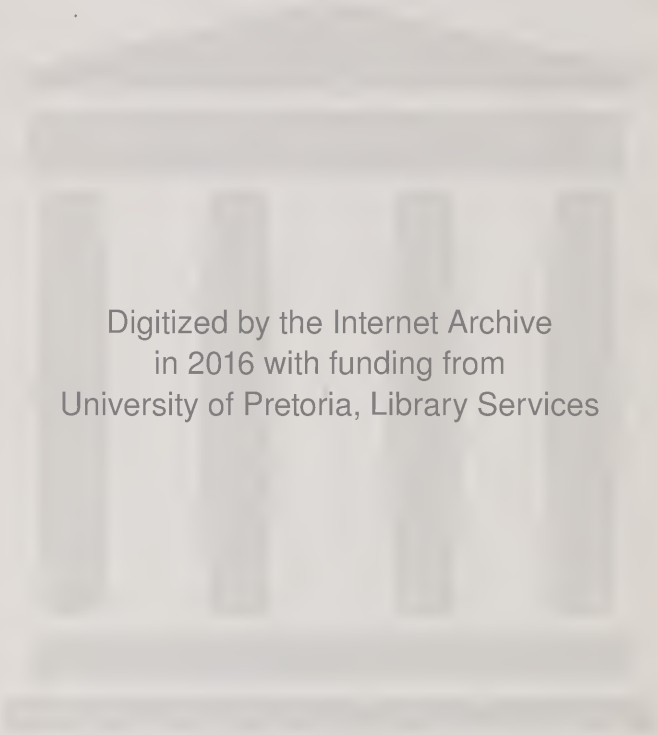


REPORT

OF THE

GOVERNMENT VETERINARY BACTERIOLOGIST.

Division of Veterinary Science,
Pretoria, 13th January, 1908.

To the Director of Agriculture.

Sir,

I have the honour to submit the annual report of the Government Veterinary Bacteriological Division for the financial year ending the 30th June, 1907, and again take the opportunity of briefly commenting on the various articles comprising my investigations during the year, pointing out the achievements and the further points to be elucidated.

(a) Further Notes on Piroplasma Mutans (N. spec.)

In my last report I described piroplasma mutans (N. spec.), a piroplasm which in former years was identified with piroplasma bigeminum of the immune ox, and a series of experiments were adduced in support of my new conception which I considered sufficient to demonstrate the duality of piroplasma mutans and piroplasma bigeminum. The results of my investigations in 1905-1906 shewed that a successful infection with piroplasma mutans was possible at any period after a pure infection of piroplasma bigeminum, and it was chiefly this fact which convinced me that piroplasma bigeminum and piroplasma mutans were two distinct species. This year a similar experiment was undertaken, but the length of time between the two infections was sufficient to exclude any possibility whatever of a retarded appearance of piroplasma mutans. For this purpose calves immune against redwater, but not against piroplasma mutans, were utilised; this fact was ascertained experimentally, in as much as I observed that an inoculation of blood into susceptible cattle was followed by the exclusive appearance of piroplasma bigeminum; susceptible cattle were, therefore, rendered immune against piroplasma bigeminum by injecting them with blood taken from a case of pure redwater infection. After an interval, ranging from 25 to 106 days, a second inoculation was made with blood containing piroplasma mutans. In every instance after the typical incubation time this latter parasite made its appearance. Continuing with my endeavours to prove beyond doubt that piroplasma bigeminum and piroplasma mutans are two distinct species, experiments were conducted with ticks. Piroplasma bigeminum is carried by blue larval ticks—the offspring of blue adult females which have been sucking blood on an immune or sick animal—and it follows that if the two piroplasms are in any way connected, the blue tick would likewise transmit piroplasma mutans; but another possibility arises that the two parasites may be of different species, and yet be carried by the same tick. Therefore, if an infestation with infected blue larval ticks be followed by the exclusive appearance of piroplasma bigeminum, and, at a later period it is possible to transmit piroplasma mutans by means of a blood inoculation to the same cattle, the result would be of considerable value in support of my conception.

Six heifers, all derived from Aliwal North, a district free of redwater, and therefore susceptible to this disease, were infested with numerous blue larval ticks, previously feeding on animals which contained both piroplasms in their blood. In all six cases the infestation of ticks was succeeded by the

exclusive appearance of piroplasma bigeminum, and in four animals spirillum appeared, which, as shewn in previous reports, is also carried by blue ticks. In no instance did piroplasma mutans appear; after a period varying from 64 to 106 days, the heifers were injected with blood of an animal immune to piroplasma mutans and piroplasma bigeminum, and as a result piroplasma mutans appeared after the typical incubation period, thus proving that this piroplasm does not belong to the life cycle of piroplasma bigeminum.

A further series of experiments were now made, somewhat on the same lines, but under the assumption that if piroplasma mutans is a species of its own, yet is transmitted by blue larval ticks, then infected ticks would transmit piroplasma mutans to animals already immune against piroplasma bigeminum. Therefore, if piroplasma mutans failed to appear after this infestation, and a subsequent injection of blood containing piroplasma mutans causes this latter parasite to appear, it would prove that the blue larval tick is not a host of piroplasma mutans. Five animals were utilised for this experiment and were rendered immune against piroplasma bigeminum by the injection of blood containing this parasite exclusively. A slight attack of redwater followed, from which the beasts recovered, but piroplasma mutans did not appear. From 49 to 54 days after this injection the five heifers were infested with blue tick larvæ, collected from animals suffering from piroplasma mutans and ordinary redwater; in no instance did piroplasma mutans appear. It now remained to note whether these five heifers were susceptible to this piroplasm. This was done by injecting blood containing both piroplasma bigeminum and piroplasma mutans, after a period varying from 56 to 67 days after the tick infestation. In all cases piroplasma mutans appeared after the typical incubation time, this result, therefore, further emphasizing the fact that piroplasma mutans cannot be identified with piroplasma bigeminum, and that blue tick larvæ are not carriers of both piroplasms.

An interesting experiment in connection with this subject commenced in 1904, when I collected blue ticks from an animal suffering from ordinary redwater and forwarded them to Professor Sir John M'Fadyean of London, where the larvæ were placed on a steer, and South African redwater was promptly produced. Professor M'Fadyean was good enough to forward blood preparations of this animal, and I identified piroplasma bigeminum; the steer recovered and was therefore immune to South African redwater. Later the steer was bled and Stockman injected some heifers in England, which he ultimately exported to this Laboratory. (I shall again refer to this strain of virus in my notes under the title Experiments with "English and South African Redwater.")

In London one of these heifers shewed a typical South African redwater reaction, and on her arrival here blood was taken and injected into a susceptible South African heifer. My previous argument equally applies in this case, namely, if piroplasma mutans belongs to the life cycle of piroplasma bigeminum, the former must appear in the injected South African heifer. However, piroplasma bigeminum appeared, and although the examinations were continued for 77 days, piroplasma mutans was never present. Continuing with this strain, the South African heifer was bled, and 10 c.c. was injected into three other South African heifers, and again piroplasma mutans failed to appear. The precaution was taken of ascertaining that these four injected animals were not immune against piroplasma mutans, and for this purpose, after the lapse of a period varying from 36 to 77 days, they were injected with blood containing both piroplasms, when, after the typical incubation time, piroplasma mutans appeared.

In my last annual report I enumerated some experiments shewing that piroplasma mutans was also found in cattle imported from Madagascar. During the past year I have frequently met piroplasma mutans in practice, and this fact may give you an indication of the importance of this piroplasm from a practical point of view.

On the 1st October, 1906, Mr. Lindsay, Government Veterinary Surgeon, Middelburg, forwarded some smears from a cow which died at "Tonteldoos," in an East Coast fever area. Microscopical examination proved the presence of endoglobular parasites corresponding to the description of piroplasma mutans, but since the animal was in an infected area reservation was made as to the cause of death, in order to make investigations amongst the herd. Smears from two healthy oxen running on the same farm were forwarded, and microscopical examination of these also revealed the presence of similar endoglobular parasites. It was now thought that this might prove to be a case of a pure infection of piroplasma mutans; accordingly, at our request, Mr. Lindsay tapped the animals and forwarded the blood, which was injected into two susceptible calves at the Laboratory. The result was that both of these calves developed piroplasma bigeminum and piroplasma mutans in succession.

A further case was brought to our notice by Mr. Evans, Government Veterinary Surgeon at Zeerust, who forwarded smears from a sick calf, at the same time reporting that East Coast fever was not suspected. The examination of the preparations revealed the presence of piroplasma mutans, and again a case of a pure infection suggested itself. Blood was obtained and injected into an animal at the Laboratory; the result was that both piroplasma bigeminum and piroplasma mutans appeared. It is a general experience that an animal infected with piroplasma mutans is also infected with piroplasma bigeminum, and although animals can easily be infected with piroplasma bigeminum exclusively, yet I have not been able to obtain a case of pure piroplasma mutans infection, and thus afford the proof of the duality of the two piroplasms in a reverse order. The experiments previously enumerated prove that the blue tick is not a carrier of piroplasma mutans, and therefore I conclude that it will only be possible to produce a case of pure piroplasma mutans infection when the tick which acts as the host has been found.

You will have noticed in my previous notes of last year that (1) no African animals have died from the injection of blood which contained piroplasma mutans and piroplasma bigeminum; (2) that all these injected animals shewed double reactions, and therefore I have a right to conclude that such animals are immune to both diseases. And this is the case, inasmuch as all animals inoculated in this manner have withstood natural exposure and no deaths were noted due to a subsequent natural infection of either of these parasites. Thus, for susceptible African cattle, the inoculation of blood of immune animals is safe, but this is not the case with imported English cattle. In the notes on this subject you will notice that one imported heifer died on the thirty-third day after inoculation of blood containing piroplasma bigeminum and piroplasma mutans—that is to say, at the time typical for both piroplasma mutans and for the second reaction of piroplasma bigeminum, with such lesions as are found in both diseases, and therefore the conclusion suggests itself that the double infection was responsible. This case can be considered on a parallel with our first experience of inoculating freshly imported English cattle in Potchefstroom some years ago, when we lost a number due to what was thought at that time to be the second reaction of redwater, but which in the light of our present experience, and from the fact

that we have noted in the surviving Potchefstroom cattle ring-shaped parasites, we must conclude that death was due to a double infection. Therefore, if we succeed in separating both infections, we may also succeed in immunising animals against both diseases, provided that the second inoculation is not performed before the animal has thoroughly recovered from the effects of the primary injection, and I can hold out some hopes of success in the near future.

In my last annual report I appended a classification of the various piroplasmoses known to me up to that time. Since then more have been described of the type of piroplasma parvum, namely, by Stephens and Christophers in Madras, by Miyajima and Shibayama in Japan, by Schein in Annam, and Bettencourt found it in a deer in Portugal. Bettencourt is of opinion that the two types of piroplasma bigeminum and piroplasma parvum represent two different genus, and therefore he proposes to give them separate names. Accordingly he creates a new name for the type of piroplasma parvum, which he designates "Theileria."

(B) Experiments with English and South African Redwater.

It would be of inestimable value if English cattle could be inoculated against South African redwater previous to exportation, since a great number—in fact, almost the majority—contract the disease after they have landed here and have been exposed to natural infection. It is only natural that in the past such attempts have been followed by disastrous results, for the reason that redwater is so thoroughly established that practically all Transvaal cattle are immune and retain piroplasma bigeminum in their blood. Consequently, immune and sick animals infect blue ticks, which in their turn transmit the disease to susceptible beasts. Accordingly the exposure of non-immune animals into a pasture where immune Transvaal cattle are running must be followed by the development of the disease in the susceptible animals. The only way out of the difficulty is to confer a certain amount of immunity on imported cattle before exposure. It is a general fact that susceptible cattle born in South Africa can be inoculated against redwater almost with impunity, but the same does not hold good with imported cattle, probably owing to the combined infection of piroplasma mutans and piroplasma bigeminum, as explained in my previous notes on piroplasma mutans.

Whilst staying in London some two years ago, Stockman and myself discussed this subject, and we thought it advisable to undertake the experiment. After my return to the Transvaal, I submitted the proposition to the then Acting Director of Agriculture, Mr. A. C. MacDonald, who supported the idea and furnished authority for the necessary expenditure. In England a disease exists, also known as redwater, which is caused by a piroplasm probably identical with piroplasma bovis of Europe. Cattle do not suffer so severely from redwater in England as is the case with our own disease in South African animals. Although we do not anticipate that the piroplasma bovis of Europe and the piroplasma bigeminum of South Africa are identical, yet they belong to the same type, and accordingly this fact gave grounds for expecting that the immunity obtained against one would protect against the other.

Stockman and myself decided to inoculate some cattle with English redwater and to expose them to the South African veld; another lot of cattle to be inoculated with South African redwater, and the remaining batch to be inoculated with both English and South African redwater. Six Ayrshire heifers were thereupon purchased on our behalf by Stockman, and

numbered Nos. 1 to 6. Of these heifers, Nos. 1 and 3 were inoculated exclusively with English redwater, Nos. 2 and 5 with South African redwater, and Nos. 4 and 6 with both piroplasmoses. The results of these inoculations were that No. 1 did not shew piroplasms and the reaction was atypical; No. 3 shewed a reaction in England, accompanied with piroplasms; Nos. 2 and 5 shewed typical reactions, together with piroplasms; the inoculation of English redwater into Nos. 4 and 6 gave negative results, and the subsequent inoculation of South African redwater produced a reaction, accompanied with piroplasma bigeminum.

Thus on first sight it would seem that South African and English redwater are not identical, but this can be interpreted in a different way. It will be noticed that three out of the four heifers did not shew reactions or piroplasms consequent on the inoculation of English redwater; therefore, in the first instance, it must be concluded that English redwater is not always inoculable. For the support of this view I quote an experiment which was performed with a view of obtaining the English strain in South African cattle for further immunisation purposes. Five African animals from Aliwal North, and therefore susceptible to ordinary redwater, were injected with blood of English heifer No. 1; four animals received the injection subcutaneously and one intrajugularly. As no result was obtained within three weeks of this inoculation, 50 c.c. blood of the same animal was injected subcutaneously into all five animals, again producing negative results. Another lot of five cattle from the same origin were inoculated with 10 c.c. blood of English heifer No. 3—the heifer which shewed piroplasma bovis in England—four animals received 10 c.c. subcutaneously and one 5 c.c. intrajugularly. In this instance nothing particular happened during the first three weeks, whereupon the animals received a second inoculation, subcutaneously, of 50 c.c. blood of the same animal, again with negative results. I refrain from drawing any conclusions with the results obtained from heifer No. 1, as in the first instance it was doubtful whether this animal was even immune against English redwater, since no piroplasms were noted in the blood and the reaction was not of a distinct character. But the interpretation of the experiment with the blood of English heifer No. 3 only allows of one conclusion, namely, that piroplasma bovis was not inoculable in our five beasts. All the animals utilised in these experiments were submitted to an inoculation with African redwater, with the result that four shewed piroplasma bigeminum, and in the other one the typical reaction, accompanied with the lesions of poikilocytosis, could only have been produced by the infection of piroplasma bigeminum. The conclusion from this experiment is that the previous inoculation with blood of an animal which in England contained piroplasma bovis did not protect against piroplasma bigeminum, and from the very peculiar behaviour of piroplasma bovis we may safely draw a further conclusion that English redwater is not always inoculable; South African redwater is easily inoculable, and therefore both diseases are not identical.

Exposure Experiments with the Imported Heifers.

Continuing on the lines of the arrangement made between Stockman and myself, the imported Ayrshire heifers were exposed on the farm "Linwood," near Pretoria. The temperatures were taken daily and the blood examined from time to time.

Heifer No. 1.—Exposed on the 5th January, 1907.—Three days after the temperature commenced to rise, reaching 106, and constantly remaining high during the next 47 days. Nothing particular was noticed in the blood

at the beginning of this reaction, but on the 35th day piroplasma bigeminum was noticed, remaining for some days, but disappeared from the 39th day. The lesions of poikilocytosis were occasionally noted, and the temperature returned to about normal on the 26th February. A second rise ensued on the 4th March, piroplasma bigeminum not being noticed, but poikilocytosis and marginal points appeared, and the animal remained very weak. Death occurred on the 17th March, with all the lesions of the sequel of ordinary redwater. The anæmia was so pronounced that the blood consisted almost entirely of basophile, polychromatic, and nucleated cells.

Heifer No. 3.—Exposed at Linwood on the 5th January, 1907.—Temperature commenced to rise on the 12th January, and then oscillated very irregularly for the next month; microscopical examination of the blood at repeated intervals failed to reveal piroplasma bigeminum, but the lesions of poikilocytosis were noted.

Recovered.

Heifer No. 2.—Exposed at Linwood on the 5th January, 1907.—Reaction commenced six days after exposure, when the temperature rose to over 106 and remained high for the following 14 days. Spirillum, basophile cells, the lesions of poikilocytosis, and marginal points were noted, but piroplasma bigeminum did not appear.

Recovered.

Heifer No. 5.—Exposed at Linwood on the 5th January, 1907. Irregular temperature noted soon after, and rose about three weeks later to a high elevation, touching 105·8; piroplasma bigeminum, the lesions of poikilocytosis, basophile granulations and polychromatic cells were present.

Recovered.

Heifer No. 4.—Exposed at Linwood on the 5th January, 1907.—This animal also shewed an irregular high temperature, reaching over 105 and as the maximum recorded, 106. Poikilocytosis, basophile granulations and spirillum were noted. Piroplasma bigeminum was not present.

Recovered.

Heifer No. 6.—Exposed at Linwood on the 5th January, 1907.—Irregular temperature noted on the 12th January, commencing with 106, maintaining high for the next 16 days and touching 106·8 on the 31st January, 1907. Basophile granulations, polychromatic cells and poikilocytosis, accompanied with marginal points were noted as the alteration in the blood. Piroplasma bigeminum was not noted, but the lesions of anæmia increased, and the animal died as the sequel of ordinary redwater.

I have already expressed the opinion that English redwater does not offer protection against our ordinary redwater. Of the four animals which had piroplasma bigeminum in their blood, due to the primary inoculation, after exposure they all more or less shewed lesions which could be identified with a new reinfection of piroplasma bigeminum. It will be seen from the experiments quoted that the inoculation of English cattle with South African redwater did not give a complete guarantee against a natural infection of piroplasma bigeminum, and we may to some extent explain the reason for this apparent failure. The animals arrived in December, almost directly afterwards they were exposed, and naturally at a time when the tick infection was at its maximum, so that they had to become acclimatised under very adverse conditions. From previous experience we know that an animal immune against redwater may break down in immunity under the influence of a subsequent heavy tick infection, and taking this fact into consideration, together

with the loss of only one English heifer, I think you will agree with me that the experiment has afforded very encouraging results. I therefore propose that a further experiment be made , but with some slight variations, namely (1) to inoculate the cattle in England with South African redwater, (2) after their arrival in the Transvaal to again inoculate them against our disease, and keep them for the first month in a paddock to ensure complete acclimatisation, and (3) to perform this experiment so that the exposure would take place at the end of the winter, when the tick infection in the veld would be at its minimum, and, with the increase of the hosts of piroplasma bigeminum, the cattle would gradually obtain a better protection against the disease, so that by December they could arrive at a complete immunity.

(c) Further Transmission Experiments of East Coast Fever by means of Ticks.

Under this heading I refer to a series of experiments, not exclusively undertaken for the purpose as indicated, but which lent themselves to various interpretations, as sundry points in the transmission of East Coast fever by means of ticks are settled to which objections have been made by some investigators, whilst others propounded new observations which I decided to control. Generally speaking the experiments were undertaken for the purpose of producing pure cases of East Coast fever on the station, in order to utilise the blood and material obtained from sick animals, for inoculation. You are aware that hitherto I have not succeeded in transmitting the disease except through the agency of ticks, and that accordingly the *sine qua non* for inoculation purposes is non-existent. I shall not enumerate all these inoculation experiments, sufficient to say that they were performed with many variations, and in anology with other diseases but they completely failed, so that for the purpose of combating East Coast fever, no recourse can be had to artificial immunisation, and the sole hope of success lays in a strict adherence to the regulations which are based on the results of our previous experience.

A few experiments were carried out in connection with the transmission of piroplasma mutans by the same agency, but I do not propose to furnish details, as in my opinion the conclusions are incomplete. I can, however, safely state that piroplasma mutans was not carried by any of the stages of the brown and blue ticks.

I wish to refer to my previous notes in the Annual Report for 1903–1904, in which I supplied the following conclusions :—

Rhipicephalus decoloratus (the common blue tick) is not a host of piroplasma parvum.

Rhipicephalus evertsi (the red tick) is not a host of piroplasma parvum.

Rhipicephalus simus is a host of piroplasma parvum.

Amblyomma hebraeum may be a host of piroplasma parvum.

Rhipicephalus appendiculatus (the brown tick) is the principal host of piroplasma parvum, and it was further stated that brown ticks transmit the disease principally in their imago stage, after having fed as nymphæ on sick beasts ; less so as nymphæ after having fed as larvæ, and not at all as larvæ originating from a mother tick removed from a beast infected with East Coast fever. These latter experiments were confirmed by Mr. Lounsbury of Cape Colony, and in 1906 he published a further series of experiments proving that other ticks, besides those mentioned above, act as hosts of piroplasma parvum, namely, rhipicephalus capensis, rhipicephalus nitens—both of which are closely allied to the brown tick, rh. nitens probably being

frequently confused with the latter—and above all rhipicephalus evertsi (the common red tick)—which I did not consider a carrier of the disease—may also act as a host.

Luhe, in Mense's "Handbuch der Tropenkrankheiten," refers to my experiments with infected blue and brown ticks, expressing the opinion that the failure to transmit the disease may be due to the fact that the larvæ were utilised too soon after they had hatched. In support of his view he refers to the experiment of Professor Koch who created a new centre of infection by spreading larval ticks on a pasture.

Professor Schilling, in the "Handbuch der Pathogenen Mikroorganismen," also does not consider my experiments of the non-transmissibility of piroplasma parvum as conclusive, and he refers to the same notes of Professor Koch.

Koch's experiment, in his own words, as published in the "Cape Agricultural Journal" for January, 1904, was as follows :—

". . . In other directions we sought for a means of communicating the disease in its virulent form. For instance, intra-ocular injections with infected blood were tried without effect and we also endeavoured by means of tick infection experiments to imitate natural methods. For this purpose cultures of the various varieties of suspected ticks were prepared. At first much difficulty was experienced in hatching out such cultures on account of the coolness and dryness of the atmosphere, conditions which experience has shewn are unfavourable for work of this description. Ultimately, however, by the use of an incubator in whose interior the humidity of the air was artificially increased, the eggs laid by ticks collected from our animals were hatched out as expeditiously as they are under the most favourable natural conditions, but when the young ticks so hatched were placed on healthy animals we found that with the exception of certain doubtful cases we failed to produce a characteristic attack. Trials were made with broods of various varieties of ticks—with broods hatched out at different temperatures, with broods kept for various periods before being placed on the animals—and this work is still being continued. To approach natural methods still more closely broods of young ticks were liberated in various localities on the grass and susceptible animals were subsequently grazed in such places. That this method should be successful appeared somewhat doubtful as we expected that the drought, high winds, dust and sun, would speedily destroy the liberated ticks, but in spite of the unfavourable weather these larval broods remained where they were placed, being most abundant on the sheltered side of the grass stems away from the sun, and particularly plentiful at the extremity of the stalks, where they clustered together in small clumps apparently waiting for the passage of a suitable host to whom they might attach themselves. These larval ticks displayed no tendency to migrate or travel from place to place, but remained where they were placed for several months. High winds seemed to scatter them a little in the direction in which the wind was blowing, but no other atmospheric change appeared to affect them. Soon after sowing these broods of seed ticks in the veld we found that it became highly infective. *Previously only occasional cases of African Coast fever had occurred* amongst animals grazing in these places, the natural veld infection appearing to be so slight that animals must graze there for many weeks without sickening, while latterly ticks had become exceedingly scarce and cases of sickness had been correspondingly few in number, apparently on account of the cold and drought." (The italics are mine.)

This experiment would tend to shew that the infection passes through the egg. Needless to say an experiment carried out in this manner cannot give forcible conclusions; *the larval ticks were distributed on an already infected area*, and with the introduction of fresh susceptible cattle the East Coast fever carrying ticks already present on the pasture, became infected and produced the broadcast infection.

It will be noticed that Professor Koch does not state in his article quoted that the blue tick was utilised, but simply mentions the fact that various varieties of ticks were used in his trials. However, at the "Conference on Diseases amongst Cattle," held at Bloemfontein in December, 1903, referring to this experiment he stated that he had only got these results with the blue tick, and therefore was of the opinion that the blue tick was of most importance in spreading the disease. He would not say that other ticks did not carry the disease, but he had never been able to produce infection with any but the blue tick. He had strong proof of his opinion, but still he would not be positive.

Our experiments were, therefore, undertaken again, in order to prove in an unmistakable way that the blue tick is not a carrier of the disease. For this purpose four animals were infested; I only utilised four animals, but the infestation was so heavy that no other interpretation is possible than that the blue tick did not act as the host of piroplasma parvum, and this experimental observation coincides with our experience in practice.

A further fact which forms the basis of our legislation is not accepted by Professor Schilling in his mentioned publication, namely, he refers to my statement that "an animal which is immune against East Coast fever does not act as a propagator of this disease," and as proof of his contention states that our view is contradicted by the history of the disease in Rhodesia. He asks the question "from which cattle the animals imported from New South Wales obtained the infection, if not from the cattle which were grazing in the neighbourhood of Beira and amongst which, as Koch has proved, were carriers of piroplasma parvum?" This statement does not of course constitute proof. The fact can, and must be, interpreted in a different way; East Coast fever was never in Beira; the Australian herd died of redwater, not of East Coast fever; they only died of East Coast fever after their arrival at Umtali. The best proof of this is that a mob of Madagascar oxen were grazing together with the Australian herd at Beira. Madagascar oxen are immune to ordinary redwater, and although a number of the Australian herd died, the Madagascar mob remained healthy. However, after the Madagascar mob were transported to Rhodesia, numbers died from East Coast fever, thus proving that these cattle were not immune to this disease. Having established this fact, it is quite clear that the disease responsible for the deaths amongst the Australian animals at Beira could not have been East Coast fever, otherwise the infection would have spread to the Madagascar herd. Therefore the infection of the Australian herd, after removal to Umtali, must have been contracted at that latter place.

I will now quote a statement made by Mr. J. M. Orpen, late Head of the Agricultural Department, Rhodesia, in the periodical "South Africa," dated March 18th, 1905, in support of my statements, and which supply the correct answer to Professor Schilling's question:—

"Before the arrival in Beira in November, 1900, of the shipment of a thousand head of cattle, which Mr. Rhodes introduced from New South Wales, as the first of an intended series of such importations by bought or chartered steamers, for the purpose of supplying the European settlers with stock, a gentleman in Umtali introduced by railway into that town a number of

slaughter cattle, which had come from German East Africa through Beira. Part of them he kept for sometime on Umtali commonage, and others he passed on by rail to Salisbury where they were slaughtered at the poles in the Makabusi Valley, on the commonage of that town. Not long after this, and just in those two places, some cases occurred of typical African Coast fever and then there began to spread from those two places along the various roads in Rhodesia a mixed infection of redwater and this new fever till then unknown to science, and indistinguishable from ordinary redwater, of which it was, of course, supposed to be only a severe form. No such outbreak occurred in the Portuguese territory through which the cattle had been brought quickly by *rail*."

"Soon after the introduction of these diseased animals, there arrived at Beira the splendid and healthy cattle from Australia which Mr. Rhodes was introducing. A breakdown occurred on the railway, and the imported cattle had, therefore, to be pastured on the Beira flats, as many others had been before. None previously pastured there had contracted African Coast fever which does not appear to exist there at all. But these Australian animals, coming from a country where no redwater exists and thus being susceptible, contracted that disease and began to die. . . . The cattle were taken to Umtali by rail and thence to a neighbouring Government farm, and isolated from all other animals. Most of them died there of ordinary redwater, but it is evident from the report (British South Africa's Company Shareholder Report for 1903), that many of them eventually died of African Coast fever, which as I have shewn had been brought to Umtali from German East Africa."

The objection raised by Luhe, certainly seemed to have some foundation. It may be that the brown tick which in the nymphal and adult stage is a carrier of piroplasma parvum, may carry it as larvæ of an infected female, when that larvæ is sufficiently old to allow for the development of the parasite. This point was also cleared up. Brown females were collected on the Coast near Durban in Natal from sick cattle, and from the same cattle brown nymphæ were also taken, which, after hatching in the Transvaal, promptly produced the disease. Bearing Luhe's observation in mind, tests were made to note if the larvæ of females taken off the same animals would transmit the disease. Altogether 19 animals were infested with brown tick larvæ. the progeny of these infected females, and the period which had elapsed after placing these ticks on the susceptible cattle and their hatching varied between 22 and 71 days. In no instance was the infestation followed by the disease and there is no reason to suppose that the animals were immune, because they all came from Aliwal North, and belonged to a group of animals of which some were utilised for infection with brown adults and promptly contracted the disease. Thus on investigation the objections of Luhe prove to be untenable, but another contingency offered itself by anology with the transmission of piroplasmoses by other ticks, namely, that the future stage in the life cycle of the tick may transmit the disease. The engorged larvæ of these infected females were therefore all collected and after moulting were placed on 20 different animals, and again after a lapse of time sufficiently long to allow for the development of the parasite. In no instance was East Coast fever observed. The next stage (*i.e.*, the imago) had also to be tested; therefore after the nymphæ had hatched they were collected and the imagines placed on five different cattle, and in no instance did East Coast fever follow.

It so happened that an animal of this batch was utilised later, in which he succumbed to the infestation of infected brown ticks, thus proving that no immunity existed due to these previous infestations.

We may safely conclude, therefore, that the brown tick which as an adult fed on sick cattle, is not a possible factor in the propagation of the disease in any of its later stages, that is to say, the infection does not pass from the female through the egg, and infect either larvæ or nymphæ.

As already mentioned, Lounsbury stated that in his experiments the red tick proved to be a carrier of the disease. My previous experiment failed, some of his failed, so that the conclusion may be drawn that red ticks do not so readily lend themselves to the propagation of East Coast fever as the brown tick. I have repeated Lounsbury's tests, obtaining my ticks from different sources, and have been able to transmit the disease to three animals, whereas a large number failed.

With regard to the bont tick, the opportunity occurred to see whether these ticks also act as hosts of piroplasma parvum. Nymphæ were collected from the Natal cattle, and after moulting in the Laboratory, were placed on healthy cattle, but no results were obtained. I also experimented with larvæ, the offspring of females sucking blood on sick cattle, these experiments also gave negative results. It now remains to be seen whether the larvæ which as nymphæ fed on sick cattle, would transmit the disease in one of its later stages.

Mr. Lounsbury was good enough to forward me some infected rhipicephalus capensis ticks, which I placed on a beast. The result was that they transmitted the disease, as Mr. Lounsbury had anticipated, but only after an incubation time of 30 days, which is very long compared with the results given by the brown tick.

The résumé of the facts mentioned in my investigations is therefore as follows :—

(1) Rhipicephalus decoloratus (the blue tick) is not a host of piroplasma parvum ;
(2) Rhipicephalus appendiculatus (the brown tick) is a host of piroplasma parvum ;
(3) Rhipicephalus evertsi (the red leg tick) is a host of piroplasma parvum ;
(4) Rhipicephalus capensis (the cape tick) is a host of piroplasma parvum ;
(5) Rhipicephalus simus is a host of piroplasma parvum ; and,
(6) according to Lounsbury, Rhipicephalus nitens is a host of piroplasma parvum.

In the article by Mr. Lounsbury, *Cape Agricultural Journal*, May, 1906, page 638, he states : " Other observers have based their determination of African Coast fever chiefly on the occurrence of a certain rod-shaped intracorpuscular organism, named by Dr. A Theiler, piroplasma parvum, in the blood of the affected animal. The almost invariable presence of this organism in blood smears from very sick animals has led to the assumption that the disease is caused by it. Mr. Robertson's observations on the experimental cases, however, throw doubt on the correctness of this assumption. He permits me to say that he has been unable to find the organism in any of the smears taken from nine of the cases, and that it has been of doubtful occurrence or rare in almost as many others. In some cases, on the other hand, it has been as numerous as in cases of the disease contracted on the veld in Rhodesia or the Transvaal, indicating that the condition necessary for its appearance in the blood is sometimes present in Cape cattle."

From this extract it must be concluded that Mr. Robertson doubts that the piroplasma parvum is the actual cause of East Coast fever ; that in other words, piroplasma parvum may be an accidental occurrence due to the

presence of East Coast fever, which latter disease may be caused by an ultra-visible micro-organism. This objection had some foundation, and as long as we experimented with cattle in South Africa it would hold good, since it may be that cattle born in a South African redwater area may contain that organism which subsequently develops under the influence of East Coast fever. As I could not undertake the experiment in South Africa, I corresponded with Mr. Stockman in London, who at once kindly consented to carry out some experiments in his Laboratory for the purpose of elucidating this point. The object was to utilise cattle which had never in any way previously been in contact with any kind of piroplasmosis infection, and this was naturally possible in England. I forwarded two batches of ticks, one of red imagines and the other brown imagines. Stockman placed the ticks on two animals, and in both cases was the infestation of the cattle followed by East Coast fever, and the piroplasms present in the blood preparations were as numerous as in any cases observed in the Transvaal. I therefore conclude that the tick which carries East Coast fever also carries piroplasma parvum, and that this piroplasm is the actual cause of East Coast fever. Mr. Robertson's observations suggest a different interpretation, and we only need refer to redwater of cattle, to the piroplasmosis in the horse, and to the trypanosomiasis in cattle, the disease may develop in the animal itself with a minimum of parasites present in the blood. Therefore cases of East Coast fever may escape microscopical diagnosis for this reason.

As mentioned before in connection with piroplasma mutans, confusion with an infection of this latter parasite may render an accurate diagnosis extremely difficult and even impossible. The fact remains, however, that so far in our experience we have not diagnosed East Coast fever and found reason to correct our verdict at a later date.

(D) Results of Horse-Sickness Inoculation in Practice, 1906–1907.

The inoculation of mules against horse-sickness was recommenced in September, 1906. In the experiments at this Laboratory I noticed that the immunity obtained by the injection of virus of the ordinary strain, did not offer a complete protection against a subsequent spontaneous infection of horse-sickness; the mortality from relapses being 0·6 per cent.; further experimental cases proved that we were able to break this immunity by the injection of a virus obtained from Tzaneen and it was expected that the immunity from this latter strain would afford a better protection against the ordinary strain than *vice-versa*. I accordingly decided to introduce this new strain into practice. This was done in September, 1906, when the necessary instructions were given to the Government Veterinary Surgeons, and up to the new year the Tzaneen virus was utilised in practice. This innovation seemed to prove satisfactory until about December, when reports came to hand, stating that the new strain did not produce the anticipated reaction. An investigation was therefore made, and the remainder of the virus in the hands of the Government Veterinary Surgeons was returned to the Laboratory, and tested on both horses and mules, the results proving that it was inert. This was contrary to all our previous experience, inasmuch as we had already ascertained that the virus could be preserved for several years without losing its virulency. This fact is therefore worth noting, but the reason cannot yet be given, although experiments to this end were immediately undertaken, and are still being continued.

It was apparent, however, that animals inoculated with the strain of virus which had proved inert, did not acquire any immunity. Fortunately

in some cases we could repair this mishap by the re-inoculation of the animals with the ordinary strain. For instance, in Rustenburg where 102 mules were inoculated with Tzaneen virus, 15 contracted horse-sickness and died, the owners being compensated, and the remaining 87 were re-inoculated free of charge; in Natal where the Tzaneen strain was also used, the re-inoculation was carried out before the animals were exposed. In Rhodesia, however, 80 mules were inoculated with the Tzaneen strain, and when our news had reached the Chief Veterinary Surgeon it was impossible for him to collect the animals or to convince the proprietors of the necessity of a second inoculation; in this case the losses after exposure were rather heavy, and amounted to about 25 per cent. With this experience at my disposal I decided after the New Year to completely discontinue the use of the Tzaneen strain and re-introduce the ordinary strain which had been used in 1905–1906.

A deviation was made from our usual practice last year, by authorising the Government Veterinary Surgeons to inoculate mules on the owners' farms whenever requested, providing they were satisfied that the proprietors would give the necessary attention during the reaction, and this arrangement proved very satisfactory, especially in cases where the farm was situated at some considerable distance from the District Office.

During 1906–1907, in the various districts of the Transvaal, 3,155 animals were inoculated with a loss of 125, or 4 per cent.; Rhodesia inoculated 972 mules with a mortality of 21, or 2·0 per cent.—these figures do not include animals inoculated with inert virus. In the Orange River Colony, 24 were treated, with one death, or 4 per cent.; Natal inoculated 1,170, and 59 died—5 per cent.; Bechuanaland was rather unfortunate, and lost 3 out of 35 inoculated; 76 mules were inoculated in Swaziland, of which 4 died. The total for South Africa was 5,432 inoculated, with a mortality of 213, or 3·9 per cent.

The total number of mules immunised in South Africa, together with the mortality, since November, 1905, is 8,766 mules inoculated—329 deaths —3·7 per cent.

In addition to these figures, the mortality after exposure enters into consideration. Of the 8,325 immunised mules, 112 died after discharge, or 1·3 per cent., but it is improbable that horse-sickness was responsible for the death in every case, although in arriving at these figures all deaths after discharge were included, and considered as horse-sickness, unless certified by the owner or the Government Veterinary Surgeon to the contrary.

We may, therefore, conclude that the total percentage of deaths amongst mules treated by our immunisation method is 5 per cent., in other words, of every 100 animals inoculated, 95 survived and successfully passed through the worst horse-sickness season experienced for many years.

As far as the statistics are to hand, the total number of deaths from horse-sickness amongst horses and non-inoculated mules during last season amounted to 6,783, and although this return is incomplete, it affords an idea of the severe nature of the disease in 1906–1907.

(E, F, AND G) THE INVESTIGATIONS INTO HORSE-SICKNESS.

In the three articles on this subject detailed in my research experiments, *i.e.* (*a*) "Further notes on immunity in horse-sickness," (*b*) "Immunisation of mules with inadequate and adequate virus and serum and the immunity obtained therefrom," and (*c*) "Inoculation of mules with polyvalent virus," I am able to add to my previous notes regarding the immunity of mules and horses against horse-sickness. I pointed out in my former report that a

certain mortality occurred amongst immunised mules, amounting to 0·6 per cent., these relapses being known to the farmer under the name of "Aanmaning." I then expressed the opinion that it is not the animal which loses its immunity, but that several strains of horse-sickness exist in the various parts of the country—and even in one and the same locality—which do not protect reciprocally. I especially referred to a virus obtained from Tzaneen, and to another from Bulawayo, the former from an immunised horse, and the latter from an immunised mule, both of which were suffering from relapses. This year the deaths after exposure amongst 8,325 immunised mules amounted to 1·3 per cent., and the object of my experiments was to detect the extent of these relapses amongst immunised mules, when subsequently tested.

In order to avoid repetition, I will detail the practice followed in all these experiments: Mules were immunised against a particular strain of virus (such as ordinary, Tzaneen, etc.), the proof of their immunity being furnished by the temperature reaction; subsequently they were tested either by an injection of a few cubic centimetres of virus or by hyperimmunisation by means of a direct transfusion of about 9 litres of virulent blood; some mules were tested with several different strains. In detailing the results I have excluded all reactions accompanied with the presence of piroplasma equi, or if the temperature reaction was absolutely abnormal for horse-sickness, and suggested that the disease was of a different nature.

The term 'Ordinary Virus" refers to the first virus used in practice and collected in Pretoria. The ordinary serum was obtained from animals injected with ordinary virus.

(1) Ordinary Virus.

A.—Mules.

I will first of all deal with the mules immunised with the ordinary virus, that is the virus used in practice during 1905–1906.

(*a*) Mules immunised with ordinary virus, and tested with ordinary virus.

273 Mules were inoculated and tested, with the result that none contracted horse-sickness, nor did any shew a horse-sickness reaction. The conclusion, therefore, is that the immunity in mules obtained by the ordinary virus protects against a subsequent inoculation or infusion of the same virus.

(*b*) Mules immunised with ordinary virus, and tested with Tzaneen virus.

139 Mules were used, with the result that 12 shewed reactions, 4 reactions with dik-kop, and 1 died; the percentage being reactions—9 per cent., reactions with dik-kop—3 per cent., and deaths—0·7 per cent.

(*c*) Mules immunised with ordinary virus, and tested with Bulawayo virus.

Of 36 animals treated and tested in this way, 7 shewed distinct reactions, 6 reactions with dik-kop, 1 doubtful reaction, and 6 deaths; the percentage being reactions—19 per cent., reactions with dik-kop—16 per cent., and deaths—16 per cent.

(*d*) Mules immunised with ordinary virus, and tested with a mixture of Bulawayo and Tzaneen virus.

Of 6 animals, immunised with ordinary virus and tested with a mixture of Bulawayo and Tzaneen virus, 2 shewed reactions, or 33 per cent.

These statistics shew in a demonstrative manner the divergency of horse-sickness and the immunity obtained therefrom.

B.—Horses.

(*a*) Horses immunised with ordinary virus, and tested with ordinary virus.

Referring to the experiments with horses, 104 were immunised with the ordinary virus, of which 88 were tested with the same virus; of these 1 shewed a reaction and dik-kop, or 1 per cent., and 3 a doubtful reaction—3·4 per cent. The one reaction with dik-kop is the only instance in which a horse immunised against the ordinary strain, has shewn a relapse with dik-kop, due to a subsequent injection of the same virus, and it is quite contrary to the result obtained from the corresponding experiment on mules. Shortly before testing, this horse was exposed at Onderstepoort, where some immunised horses and mules had contracted the disease spontaneously, so that it is quite possible that the horse was not suffering from a relapse due to the test, but to a natural infection of a different strain of virus.

(*b*) Horses immunised with ordinary virus, and tested with Tzaneen virus.

40 Horses immunised with ordinary virus were tested with Tzaneen virus, producing 12 reactions—30 per cent.; 6 reactions with dik-kop—15 per cent; 1 doubtful reaction, probably due to piroplasma equi, and 7 deaths—17 per cent.

(*c*) Horses immunised with ordinary virus, and tested with Bulawayo virus.

26 Horses were immunised with ordinary virus, and tested with Bulawayo virus, the result being that 3 shewed reactions—12 per cent.; 4 reactions with dik-kop—16 per cent; 1 doubtful reaction, and 5 deaths—20 per cent.

(*d*) Horses immunised with ordinary virus, and tested with a mixture of Ordinary, Tzaneen and Bulawayo virus.

Of 10 horses immunised with ordinary virus, and tested with a mixture of O–T–B virus, none contracted horse-sickness or died.

(*e*) Horses immunised with ordinary virus, and tested with virus obtained from spontaneous cases.

43 Horses were immunised with ordinary virus, and tested with virus obtained from spontaneous cases in the Zoutpansberg district, of which 2 shewed reactions—5 per cent.; 3 doubtful reactions, and 1 death—2 per cent.

In comparing the results obtained from immunised mules with immunised horses when tested with various vira it will be seen that only 1·5 per cent. of immunised mules died, whereas the mortality in immunised horses amounted to 6·2 per cent.

(2) Tzaneen Virus.

A.—Mules.

In order to overcome the possibility of subsequent relapses in immunised mules, and observing that the immunity obtained by the ordinary virus did not completely protect against an injection of the Tzaneen strain, the latter virus was introduced into practice last year. Control experiments were then carried out to note the protection it afforded against the other virus.

After it was ascertained that the mortality due to the simultaneous inoculation of mules with Tzaneen virus and serum obtained from animals hyperimmunised with the ordinary strain, was not higher than when ordinary virus was used in conjunction with ordinary serum, this alteration in the method was considered safe, and virus of various generations of the Tzaneen strain was utilised in conjunction with ordinary serum.

(*a*) Mules immunised with Tzaneen virus, and tested with Tzaneen virus.

23 Mules were immunised with the Tzaneen strain (*i.e.*, Tzaneen virus and ordinary serum) and tested with the ordinary virus, with the result that 18 gave reactions—70 per cent.; 2 reactions with dikkop—9 per cent, and 3 deaths—11 per cent. It therefore appeared that the immunity obtained fro n the Tzaneen virus in conjunction with the serum of the ordinary strain, did not protect against a subsequent injection of the ordinary virus, in the same way as the immunity obtained from ordinary virus and serum resisted the Tzaneen virus.

The interpretation of this fact is probably that in inoculating mules with the ordinary virus and adequate serum, a complete immunity is obtained against the virus, but in using ordinary serum against Tzaneen virus—the virus and serum being inadequate—it is probable that not all the components of the Tzaneen strain are capable of making an impression on the system of the mule; in other words, the ordinary serum nullifies the effect of certain components of the Tzaneen strain, and consequently does not give complete immunity. This conception has a support in the following:—

(*b*) Mules immunised with Tzaneen virus and ordinary serum and tested with Tzaneen virus.

26 Mules were immunised with the Tzaneen strain, and the test with the Tzaneen virus produced 19 reactions, or 73 per cent. The test was made with a higher generation of virus than that used for the immunisation, and possibly this may account for the relapses: but the fact can also be explained if we accept that the Tzaneen virus is already a complex virus, and the serum being of the ordinary strain neutralises some components of the Tzaneen virus and prevents the formation of antibodies in the injected animals which are active against the whole of the Tzaneen virus. When at a later period the effect of the serum has passed over and the same virus is again utilised, those components which could not produce anti-bodies are now able to cause a reaction.

It has, therefore, to be expected—as is the case with the ordinary virus—that the immunity obtained from a simultaneous injection of Tzaneen virus and the adequate serum would afford a better protection than that given by the injection of Tzaneen virus and ordinary serum.

(*c*) Mules immunised with Tzaneen virus, and tested with Bulawayo virus.

Of 11 mules immunised with the Tzaneen virus and ordinary serum, and tested with Bulawayo virus 5 gave reactions—44 per cent.; 2 doubtful reactions were noticed, and 1 death—9 per cent.

(3) Polyvalent Virus.

With all these particulars at my disposal I decided to experiment with a view of obtaining an immunity by a single injection which would protect against all three vira, namely, Ordinary, Tzaneen, and Bulawayo.

(In the following notes certain symbols, such as OTB and O-T-B appear, which must be explained. A virus or serum obtained by mixing the three strains, Ordinary, Tzaneen, and Bulawayo, was, for the sake of brevity, designated O-T-B. But if the three vira were consecutively injected and at the height of the fever reaction the animal was tapped and the blood utilised as virus, this trevalent virus was called OTB.

Thus the symbol OTBLPW means that eight different vira, Ordinary, Tzaneen, Bulawayo, Lydenburg, Piet Retief, Pietersburg (2), and Warmbaths were consecutively injected and an octovalent virus obtained from the animal during the fever reaction.)

(*a*) Virus O–T–B and Serum O–T–B.

Injections with this virus and serum produced reactions in all cases, but when the animals were subsequently tested with any of the constituents of the virus mixture separately, it appeared that the immunity was not complete.

A further experiment was made by injecting mules with a virus composed of the mixture of two virus, either ordinary and Tzaneen, or ordinary and Bulawayo, or Tzaneen and Bulawayo, the serum in each case being the mixture O–T–B. Again reactions were produced, but a subsequent test with either components of the virus mixture produced relapses.

These two failures must similarly be interpreted by admitting that the mixture of the various sera did not represent the adequate serum to the virus mixture, and one or the other virus was precluded from having any effect on the mule.

(*b*) Experiments with Virus OTB and Serum O–T–B.

The three strains of virus were now consecutively injected into a horse, and when the horse-sickness reaction ensued the horse was tapped and the blood utilised as virus. This virus was expected to be trevalent. The serum was obtained by mixing the sera corresponding to the three strains.

The simultaneous injection of this virus and serum into mules, produced reactions in all cases and immunity was apparently established, but when the animals were subsequently tested with one of the component strains, an unmistakable breakdown was noticed, probably for the same reason as explained previously.

Polyvalent immunity cannot therefore be obtained in this way.

(*c*) Virus OTBLPW and Serum O–T–B.

This virus was obtained on the same lines as in the previous case, but with eight different strains of virus obtained from Ordinary, Tzaneen, Bulawayo, Lydenburg, Pietersburg (two kinds), and Piet Retief.

The serum was O–T–B.

Again all injected mules reacted, but a test with any of the components of the virus mixture produced breakdowns.

(*d*) Virus OTB and Serum OTB.

The final experiments were now made by immunising animals with polyvalent virus and serum.

The virus used was OTB (*vide* Experiment 3*b*), and to which the serum corresponded. The result was that immunity given by virus OTB and serum OTB, with one exception, protected against a subsequent injection of any of the components of the virus mixture.

(*e*) Virus OTBLPW. Serum OTBLPW.

The simultaneous injection of virus OTBLPW (*vide* Experiment 3*c*) and a corresponding serum, did not completely protect against a subsequent injection of any of the components of the virus, but in the majority of cases no breakdowns were noted.

In comparing these results it will be seen that immunity obtained by the polyvalent virus and serum afforded a better protection against the constituents of the polyvalent virus than the immunity obtained by an injection of a mixture of the various virus and their adequate sera (O–T–B).

(h) Continuation of Experiments for Inoculation against Equine Piroplasmosis.

In my last annual report a number of experiments were enumerated shewing that (1) the inoculation of mules with immune mule blood can be performed with a large prospect of success; (2) that a certain amount of risk is attached to the inoculation of donkeys with immune mule blood, and (3) that the inoculation of horses with immune donkey blood may be followed by disastrous results. During the past year these experiments have been continued on a somewhat different line, and based on the observation made in connection with redwater, namely, that the inoculation of cattle with blood of a calf immune against this disease is not so frequently followed by strong reactions and mortality as when the blood is derived from a full-grown beast.

Accordingly I decided to utilise the blood (*a*) from young immune weaned horse foals, and (*b*) from immune donkey foals which were still suckling. The experiments were classified according to the origin of the blood, namely, (1) from an immune horse, (2) from an immune mule, and (3) from an immune donkey. The various strains of virus were subsequently passed through horse foals and donkey foals, and blood of each generation used for inoculation purposes.

Four Transvaal horse foals were injected with blood from an immune horse and none died. In the second generation with this strain, eight Argentine horses were inoculated and five died; two horses were suffering from gangrenous pneumonia, probably the result of a disease contracted on board ship, called "ship's pneumonia," and died; one died from horse-sickness contracted spontaneously, and the other two were pregnant mares, and this fact, in conjunction with the piroplasmosis reaction, must be held responsible.

The second series of experiments refers to blood originating from an immune mule. The strain was passed through horse foals and continued for eight generations. The results were that five Argentine horses were inoculated and four died; 67 Argentine mules inoculated with one death, and 12 Argentine donkeys with one death; nine Transvaal horse foals and five Transvaal mules with no deaths.

With regard to the four Argentine horses which died, two were in very poor condition previous to inoculation, and death was caused by poverty complicated with piroplasmosis. A third animal died from syncope, and in the fourth instance broncho-pneumonia was responsible. The Argentine donkey slipped her foal and died, as a result of the complication with poverty and debility. The death of the Argentine mule was due to pneumonia, probably "ship's pneumonia".

Referring to the third class, where the strain emanates from a donkey, this was passed through a horse, horse foals, and donkey foals, and continued for six generations; in each generation blood from the horse foal or donkey foal was utilised with the result that

of 12	Transvaal	horse foals	inoculated ..	..	1 died
5	,,	donkey foals	,, ..	..	0 ,,
1	,,	horse	,, ..	..	0 ,,
7	,,	mules	,, ..	..	1 ,,
23	Argentine	horses	,, ..	..	3 ,,
62	,,	mules	,, ..	..	2 ,,
25	,,	donkeys	,, ..	..	0 ,,

The Transvaal mule died as a result of undoubted piroplasmosis; two Argentine horses died from rupture of the spleen; the third one was killed on account of pleuro-pneumonia. Of the two Argentine mules, one died of pure piroplasmosis and the other from the sequel of Piroplasmosis.

The conclusions, therefore, are that: (1) mares heavy in foal should not be inoculated; (2) animals in poor condition must not be inoculated; (3) animals imported from oversea should not be inoculated until all danger of an infection with ship's pneumonia has been removed; and (4) the contingency must always be expected that Argentine horses and mules may die of rupture of the spleen, as they are very wild and stabling often causes them to contract serious injuries.

Therefore, equines can now be inoculated against piroplasmosis with a small risk of mortality, providing the precautions previously mentioned are observed; and later I shall ask for your authority to introduce this method of inoculation into practice.

(i) Inoculation against Blue Tongue, and Results in Practice.

In the article "Blue Tongue in Sheep," included in my annual report for 1904–1905, I enumerated several experiments carried out on the lines proposed by Mr. Spreull, M.R.C.V.S., of the Cape Colony, namely, a simultaneous inoculation of serum and of virus in certain proportions. In my experiments the dose of serum was probably too high, and the reaction necessary to produce an active immunity escaped notice. I therefore concluded that the serum had a very strong preventive action, and for future experiments proposed to reduce the quantity of serum injected. In continuing the experiments accordingly, and infecting sheep with virus for the purpose of hyperimmunisation, I noticed that after the virus had passed through several generations the mortality from inoculation completely ceased. All the animals had reactions typical for blue tongue, which, to judge from the temperature charts, were very severe, and yet they did not shew any clinical symptoms, so that the illness was hardly noticeable. The results of these observations are that of the first 10 generations, 10 sheep died out of 93, and from the 11th to 18th generations, none died out of 319; thus, out of a total of 412 sheep, 10 died, or 2·5 per cent. After I had ascertained that the immunity from this reaction was equal to that obtained from a natural attack, I decided to introduce the inoculation into practice and to utilise the virus obtained from the 11th to 18th generations. Accordingly in February, 1907, instructions were issued to all Government Veterinary Surgeons to inform the farmers that a vaccine for the prevention of blue tongue was ready, and would be issued free of charge, together with a syringe, as far as these instruments were available. Provision was also made for the full instructions to be thoroughly explained to the farmers, so that if they wished they could perform the operation themselves.

At the commencement of the inoculation a slight mishap was experienced owing to the rain, when mud entered into the puncture caused by the inoculation, causing blood poisoning, but steps were immediately taken to warn all concerned, and further instructions issued not to inoculate during the heavy rains, when the sheep would have to be exposed to wet pastures and muddy kraals. This is an important fact, and I have decided to recommend that the inoculation for the ensuing season be started before the rains have thoroughly set in.

The total number of doses issued in the Transvaal amounted to 31,087.

Towards the conclusion of the blue tongue season a circular was issued asking the various Government Veterinary Surgeons to obtain information regarding the success of the vaccination. These returns were unsatisfactory, as only 20 per cent. of the results came to hand, shewing that 5,875 sheep were inoculated in the Transvaal, and that 74 died within 14 days after the inoculation. It must be explained here that the whole course of the disease averages 14 days; therefore this period must be allowed for the reaction, consequent upon the vaccination, before immunity is finally established; it is, therefore, quite possible that an animal suffering from blue tongue, or naturally infected on the day of inoculation, would die within this period. Consequently deaths within 14 days after vaccination must be excluded, since the animals had not acquired immunity; neither can these deaths be considered as a result of the inoculation, since we have shewn in our experiments that out of 319 animals vaccinated, none died. The percentage of deaths occurring after 14 days from inoculation—breakdowns in immunity—is about 0·6 per cent. At the same time farmers were asked to furnish returns of mortality amongst their non-inoculated sheep. These returns shew that out of 16,218 sheep, 1,817 died, an average of 11 per cent. These figures speak for themselves and testify to the success of our vaccination method.

Generally speaking, last season was a very bad one for blue tongue in sheep; a complete return of the losses are not at my disposal, but in the Bethal District 5,076 sheep died; Lichtenburg shews deaths amounting to 7,096, out of a total number of 85,047.

The farmers are unanimous in testifying to the efficacy of the vaccine, and the following extract from the report of the Resident Magistrate, Ermelo, may be taken as a fair example:—"Mr. Turner, of Clifton, inoculated the whole of his flock (1,351) with the blue tongue vaccine, with apparently excellent results, as very few of his sheep have been lost, and since the inoculation the disease has apparently died out, whereas his neighbours are still losing sheep in considerable numbers."

From previous experiments, a certain amount of blue tongue serum was at my disposal, and which, as I considered it would be waste to throw away, I decided to utilise for curative purposes in practice. This curative treatment was also mentioned in the circular to Government Veterinary Surgeons, and over 1,000 doses were issued. I did not anticipate any good results from the curative treatment of blue tongue by means of serum, as I was fully aware that the injection of serum would, in the majority of cases, be too late to have any beneficial effect. To my great surprise, however, numerous reports came to hand stating that the serum proved successful in such cases where the disease was not too far advanced, and even in some cases where the animals were very ill. On the other hand, negative reports also came to hand.

However, I have decided not to prepare any more serum, as the preparation is very expensive, and the dose required extremely high, so that the charge would be prohibitive for the common sheep, and further, seeing that the vaccination is a complete success, no curative treatment should be required if the farmer takes the precaution of inoculating his flock before blue tongue makes its appearance.

I have not yet been able to ascertain how long the immunity obtained from either a natural attack or from our inoculation will last; I know that the immunity conferred by the vaccine may be broken down by a large dose of virus, but the immunity in practice seems to be sufficiently strong to protect against and prevent any great losses, and certainly seems to protect for at least a year.

For the future, therefore, only vaccine will be prepared, and for the ensuing year arrangements have been made for meeting all requirements.

Miscellaneous Investigations.

(j) Gouw-Ziekte.

At repeated intervals during 1904–1906, Mr. Walker, Government Veterinary Surgeon at Ermelo, reported the appearance of a disease which was playing havoc amongst sheep in the New Scotland area. This disease is described under the name of gouw-ziekte (a quick sickness), and is so called from the fact that the animal is not noticed to be ill, or if so only for a very short time previous to death. Similar reports of individual cases were received from various parts of the Transvaal, including Pretoria, but I am under the impression that in these instances probably a number of different ailments are classified under this name; the fact remains, however, that a real epidemic occurs amongst various flocks after their introduction to certain farms in the New Scotland area. Mr. Walker made a thorough investigation on several farms, more especially on the farm of Mr. Dell, Mount Denny, where he carried out a series of post-mortem examinations, without coming to any definite conclusion as to the seat of the pathological lesions. Mr. Walker forwarded specimens of blood from various sheep which had died of the disease, for the purpose of inoculation experiments at the Laboratory. In all cases the microscopical examinations of the blood preparations gave negative results; the blood was injected, and care was taken to select sheep of various ages, sexes, breeds, conditions, etc., and in no case did a reaction follow, so that we may draw the conclusion that either the disease is not an inoculable one or that the virus had become inert during transmission from Ermelo to Pretoria. The former supposition, however, seems to be correct, since Mr. Walker himself inoculated some sheep with fresh blood, without obtaining any result. Arrangements were also made to obtain food from the stomachs of some of the dead animals, and the dry material was administered to sheep in the Laboratory—again with negative results.

At the request of Mr. Walker, who visited Pretoria for the purpose of furnishing us with details of the serious character of the epidemic, Mr. Gray and myself decided to make an investigation *in loco*. We were unfortunate, however, during our visit not to see any dead animals, but we obtained some good information, which led us to believe that we had to deal with a specific disease. Apparently healthy animals, which are feeding up to the last minute, are suddenly seized by a fit, jump, and drop dead on the ground. On one farm in 1905 the disease killed 100 sheep; on a second farm 60, on a third 30, and on several other farms a good many, the exact number not being known. It was noticed that in one instance the sheep commenced to die after they had been introduced to low-lying farms, and the mortality continued for three weeks after their removal to another place, when the disease died out. Principally ewes, heavy in lamb, were attacked, and out of 160 animals only four wethers and two rams died, but this may be due to the percentage of females as compared with the number of wethers and rams. Another remarkable fact is that the herd in which 100 died was chiefly composed of crossed Persian-Africanders; on the other hand, the herd in which the mortality amounted to 60 was mostly composed of crossed Africander-Merinos. On one farm 16 oxen were reported to have died from the same disease, but unfortunately no diagnosis was made, as the Government Veterinary Surgeon happened to be on leave. We searched for poisonous plants, but failed to find any, although from the information gathered, we do not consider that such plants are the cause of the mortality, but that gouw-ziekte is a specific

disease, and this conception is confirmed by the fact that sheep continue to die up to three weeks after removal to another place, thus suggesting that three weeks represents the average incubation period of gouw-ziekte.

In view of the heavy mortality due to gouw-ziekte, arrangements were made for the establishment of an experimental camp in the infected area, and Mr. Dodd was detailed to be in charge. It was intended that a thorough bacteriological examination should be carried out, and I hoped that the results would enable us to successfully combat the disease, but unfortunately at the very last moment Mr. Dodd contracted enteric, and, as I could not spare another official, the experiment had to be abandoned.

I hope that during the ensuing year, however, this investigation will be commenced.

(k) Stiff Sickness, or the Three Days' Sickness.

At the beginning of the year Mr. Gray, our Principal Veterinary Surgeon, received information from Rhodesia to the effect that a new disease was noticed in that country, attacking cattle, and causing an illness characterised by a temporary paralysis and a fever, both of which passed off in about three days' time. Blood preparations were forwarded to us by Government Veterinary Surgeon Edmonds, of Rhodesia, from various cases which had come under his observation, and were examined at this Laboratory with negative results. Soon afterwards reports came to hand detailing similar outbreaks in the Transvaal, and microscopical examinations were again without result. About February, 1907, the sickness was noticed in the neighbourhood of Pretoria, and also appeared amongst our cattle at Onderstepoort, when we were able to examine several cases. Our observations are to the effect that the disease commences with a high fever, reaching as high as 105 F.; the animal ceases feeding and ruminating; there is a slight dripping from the mouth, grinding of the teeth, and injection of the mucous membranes of the eyes. The characteristic of the disease, however, is stiffness, which attacks one or more legs simultaneously or alternately. The animal lags whilst grazing and finally lays down; once on the ground it experiences great difficulty in rising again, and, judging from its position—which resembles that assumed by a horse suffering from laminitis—all movements appear to be accompanied with acute pain. In other cases assistance, or blows, fail to enable the animal to stand, but in every instance all these symptoms disappear after about three or four days. In some animals a slight "blowing-up" was noticed (tympanitis); some shewed constipation before recovery was complete. Only one opportunity occurred of making a post-mortem examination, and this was on a cow which died near Pretoria. This was an aged beast, which at the same time was suffering from a chronic pericarditis of a traumatic nature, but not sufficient to cause death, so that the complication with stiff sickness must be accounted responsible. In the post-mortem examination the intermuscular tissue, especially of the loins and of the hindquarters, were infiltrated with serous fluid. The cause of the disease could not be ascertained by microscopical examination or by culture, but advantage was taken of the outbreak at Onderstepoort and a sick animal was tapped. The defibrinated blood was brought to the Laboratory—where the disease has not ever appeared—and 20 c.c. were injected into a young heifer on the 24th February, 1907. After an incubation time of three days a reaction ensued, the temperature reaching as high as 105 F., and continuing for about eight days. During this period the animal shewed the typical characteristic of stiffness, just as described, but as usual the symptoms suddenly disappeared. Saliva from the Onderstepoort animal was also collected by saturating cotton wool, and

subsequently rubbed on to the gums of another heifer, without any result. It may, therefore, be concluded that stiff sickness is an inoculable blood disease. With regard to its spread, observations to hand shew that almost in every instance no direct communication occurred between sick and healthy animals previous to the infection of the latter, so that pure contagion can be excluded. Our assumption is that the infection must pass through the air, and we can hardly believe that the micro-organism itself can do so, but that it will probably require a carrier, as is the case in other diseases. Taking into consideration the extremely wet season, with the enormous increase of mosquitos and other flying insects, it is quite possible that one of these may be the cause of the propagation.

In the Transvaal fortunately stiff sickness was quite of an ephemeral nature, and did not cause any serious damage.

(L) Internal Parasites.

In the train of the heavy rainfalls experienced last year an unusual mortality occurred amongst small stock—apart from blue tongue—due to internal parasites. Enquiries reached us from many parts of the Transvaal, pointing out the unusual increase of wire-worms (strongylus contortus), and asking for advice, and in several cases specimens and carcases were forwarded for examination. Besides these worms, oesophagostoma columbianum were present to a large extent, and the intestines of some sheep were studded with an abnormal number of parasitic nodules.

Internal parasites undoubtedly play an important rôle as the cause of debility and death amongst small stock, and the moist conditions of the soil during the past year were naturally extremely favourable for their increase.

I am convinced that an increase of small stock will be attended with a corresponding increase of internal parasites, and, in order to ensure the success of this class of stock breeding, it will be necessary to undertake a thorough investigation into the life cycle of each individual class of parasites.

This will only be possible by experimenting on scientific lines, and a man with a zoological training will be required to undertake the study at this Laboratory under my instructions. On the Estimates for 1908–1909, therefore, I shall apply for the appointment of a duly qualified assistant.

Microscopical and Pathological Anatomical Examinations.

With regard to the microscopical and pathological examinations performed at the Laboratory during the last year, the total number of examinations amounted to 1,597; an increase of 486 on 1905–1906. The negative results amounted to 983, and as in previous years the bulk of the examinations were in connection with the "Proclaimed Diseases of Animals Act," East Coast fever in particular. I wish to refer you to my last annual report for the explanations of the various results.

This increase shews the growing confidence of farmers in the work of this Division, and as far as my experience is concerned, I can safely say that no mistake has yet been made in a diagnosis.

It is noteworthy to mention, that amongst the negative results, two are included which were obtained from smears forwarded by natives in the Zoutpansberg district, said to be from cattle, but the examination proved the blood to be that of a bird. An investigation was made by the South African Constabulary, but without being able to prove any wilfulness on the part of the natives, although undoubtedly it was a trick played on us.

The details of the examinations are as follows :—

Summary of Microscopical and Pathological Anatomical Examinations made during the Financial Year 1906–1907.

East Coast fever, piroplasma parvum	Cattle	133
Doubtful East Coast fever	,,	3
Piroplasma mutans	,,	30
Ordinary redwater, piroplasma bigeminum	,,	30
East Coast fever and ordinary redwater, piroplasma parvum and piroplasma bigeminum	,,	5
Marginal points, a probable sequel of ordinary redwater	,,	7
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	,,	40
Poikilocytosis, a probable sequel of ordinary redwater	,,	47
Abscess due to bacterial infection	Calves	6
Abscess due to coccus infection	Equines	1
Acariasis, Sarcoptes of Africander goats	Goats	107
Acariasis, Psoroptes of sheep	Sheep	48
Acariasis	Equines	2
Amphistomum conicum	Sheep	1
Anaemia, Basic cells, ? sequel of blue tongue.. ..	,,	1
Angioma	Cow	1
Anthrax	Cattle	13
Ascaris swillæ	Pigs	1
Black quarter	Cattle	5
Broncho-alveolitis	Mule	1
Broncho-pneumonia..	Cattle	3
Broncho-pneumonia..	Pigs	1
Coryza	Horse	1
Cysticercus cellulosoe ? swine plague	Pigs	1
Cysticercus tenuicollis	Sheep	2
Diphtheria	Fowls	1
Enteritis	,,	1
Enteritis	Cattle	1
Enteritis and gastritis	Sheep	1
Epizootic lymphangitis, Saccharomyces farciminosus ..	Equines	33
Glanders	,,	16
Leucæmia	Dog	1
Mastitis	Cow	2
Necrosis	,,	1
Oesophagostoma columbianum	Sheep	1
Parasitic nodules	Horse	1
Parasitic nodules	Cattle	4
Parasitic pneumonia	Goats	1
Parasitic pneumonia	Cattle	1
Piroplasma canis	Dogs	4
Piroplasma equi	Equines	16
Poikilocytosis, sequel of Piroplasma equi	,,	1
Pleuritis	Horse	1
Pleuritis	Cattle	1
Pleuritis necrotica	Sheep	1
Pleuro-pneumonia	Cattle	5
Pneumonia	,,	1
Pneumonia	Equines	1
Septic metritis	Cattle	2
Carried forward		588

Brought forward		588
Staphylococcus	Cattle	1
Staphylococcus	Sheep	1
Stilisia hepatica	,,	1
Strangles (streptococcus equi)	Equines	1
Streptococci infection	Calves	1
Streptococci infection	Equines	5
Streptothrix	Cow	1
Streptothrix	Mule	1
Strongylus contortus	Sheep	1
Subcutaneous emphysema	Chick	1
Swine fever	Pigs	2
Traumatic hæmorrhage	Kidney of Chicken	1
Traumatic pericarditis	Cattle	3
Tuberculosis	,,	5
Vaginitis	Cow	1
Negative results	—	983
		1,597

Results of Examinations Classified according to Districts.

Barberton.

East Coast fever, piroplasma parvum	Cattle	9
Piroplasma mutans	,,	6
Ordinary redwater, piroplasma bigeminum	,,	7
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	,,	6
Poikilocytosis, a probable sequel of ordinary redwater ..	,,	6
Abscess due to coccus infection	Equine	1
Angioma	Cow	1
Mastitis	,,	1
Negative result	—	45
		82

Ermelo.

East Coast fever, piroplasma parvum	Cattle	2
Ordinary redwater, piroplasma bigeminum	,,	1
East Coast fever and ordinary redwater, p. parvum and p. bigeminum	,,	1
Marginal points, a probable sequel of ordinary redwater	,,	1
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	,,	4
Poikilocytosis, a probable sequel of ordinary redwater ..	,,	2
Abscess due to bacterial infection	Calves	1
Ascaris swillæ	Pigs	1
Epizootic lymphangitis, Saccharomyces farciminosus	Equines	4
Piroplasma equi	,,	6
Strangles (Streptococcus infection)	,,	1
Streptococci infection	,,	3
Streptothrix	Cow	1
Tuberculosis	Cattle	1
Vaginitis	Cow	1
Staphylococcus	Sheep	1
Negative results	—	68
		99

Heidelberg.

Ordinary redwater, piroplasma bigeminum	Cattle	4
Basic, nucleated and polychromatic cells a probable sequel of ordinary redwater	,,	2
Poikilocytosis, a probable sequel of ordinary redwater	,,	4
Acariasis, psoroptes of sheep	Sheep	1
Broncho-pneumonia	Cattle	1
Glanders	Equines	2
Parasitic nodules	Horse	1
Pleuritis	,,	1
Coryza	,,	1
Negative results	—	31
		48

Johannesburg.

Ordinary redwater, piroplasma bigeminum	Cattle	3
Acariasis, sarcoptes of Africander goats	Goats	5
Acariasis, psoroptes of sheep	Sheep	9
Anthrax	Cattle	5
Broncho-pneumonia	Pigs	1
Enteritis and gastritis	Sheep	1
Epizootic lymphangitis, saccharomyces farciminosus	Equines	22
Glanders	,,	8
Swine fever	Pigs	2
Traumatic hæmorrhage	Kidney of Chicken	1
Streptothrix	Mule	1
Negative results	—	85
		143

Krugersdorp.

Acariasis, psoroptes of sheep	Sheep	4
Glanders	Equines	1
Pleuro-pneumonia	Cattle	4
Traumatic pericarditis	,,	1
Negative results	—	21
		31

Lydenburg.

East Coast fever, piroplasma parvum	Cattle	7
Ordinary redwater	,,	1
Marginal points, a probable sequel of ordinary redwater	,,	1
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	,,	2
Poikilocytosis, a probable sequel of ordinary redwater	,,	3
Epizootic lymphangitis, saccharomyces farciminosus	Equines	4
Negative results	—	28
		46

Marico.

Piroplasma mutans	Cattle	2
Poikilocytosis, a probable sequel of ordinary redwater	,,	2
Acariasis, sarcoptes of Africander goats	Goats	1
Negative results	—	22
		27

Middelburg.

East Coast fever, piroplasma parvum	Cattle	10
Piroplasma mutans	,,	7
Ordinary redwater	,,	4
Marginal points, a probable sequel of ordinary redwater	,,	1
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	,,	5
Poikilocytosis, a probable sequel of ordinary redwater	,,	1
Abscess due to bacterial infection	Calves	1
Acariasis, sarcoptes of Africander goats	Goats	2
Acariasis, psoroptes of sheep	Sheep	2
Broncho-pneumonia	Cattle	1
Mastitis	Cow	1
Oesophagostoma columbianum	Sheep	1
Pleuritis	Cattle	1
Negative results	—	69
		106

Piet Retief.

East Coast fever, piroplasma parvum	Cattle	7
Doubtful East Coast fever	,,	2
Piroplasma equi	Equines	1
Negative results	—	15
		25

Potchefstroom.

Ordinary redwater, piroplasma bigeminum	Cattle	1
Acariasis, psoroptes of sheep	Sheep	2
Amphistomum conicum	,,	1
Anthrax	Cattle	1
Pleuritis necrotica	Sheep	1
Tuberculosis	Cattle	1
Negative results	—	20
		27

Pretoria.

East Coast fever, piroplasma parvum	Cattle	1
Piroplasma mutans	,,	1
Ordinary redwater, piroplasma bigeminum	,,	7
Marginal points, a probable sequel of ordinary redwater	,,	2
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	,,	11
Poikilocytosis, a probable sequel of ordinary redwater	,,	12
Abscess due to bacterial infection	Calves	3
Acariasis, sarcoptes of Africander goats	Goats	19
Acariasis, psoroptes of sheep	Sheep	17
Anthrax	Cattle	3
Necrosis	Cow	1
Broncho-pneumonia	Cattle	1
Cysticercus tenuicollis	Sheep	1
Diphtheria	Fowls	1
Enteritis	..	1
Carried forward		81

Brought forward		81
Enteritis	Cattle	1
Epizootic lymphangitis, saccharomyces farciminosus ..	Equines	2
Glanders	,,	4
Cysticercus cellulosoe	Pigs	1
Leucaemia	Dog	1
Parasitic pneumonia	Goats	1
Parasitic pneumonia	Cattle	1
Piroplasma canis	Dogs	3
Pleuro-pneumonia	Cattle	1
Pneumonia	,,	1
Septic metritis	,,	2
Streptococci infection	Equines	2
Strongylus contortus	Sheep	1
Subcutaneous emphysema	Chick	1
Traumatic pericarditis	Cattle	2
Tuberculosis	,,	3
Stilisia hepatica	Sheep	1
Staphylococcus	Cow	1
Negative results	—	237
		347

Rustenburg.

East Coast fever, piroplasma parvum	Cattle	35
Doubtful East Coast fever. ? Piroplasma mutans ..	,,	1
Ordinary redwater, piroplasma bigeminum	,,	1
East Coast fever and ordinary redwater, piroplasma parvum and piroplasma bigeminum	,,	2
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	,,	4
Poikilocytosis, a probable sequel of ordinary redwater ..	,,	4
Anthrax	,,	4
Black quarter	,,	3
Streptococci infection	Calves	1
Negative result	—	74
		129

Standerton.

Poikilocytosis, a probable sequel of ordinary redwater ..	Cattle	1
Abscess due to bacterial infection	Calves	1
Acariasis, psoroptes of sheep	Sheep	3
Epizootic lymphangitis, saccharomyces farciminosus ..	Equines	1
Glanders	,,	1
Negative results	—	19
		26

Volksrust.

Poikilocytosis, a probable sequel of ordinary redwater ..	Cattle	1
Acariasis	Equines	2
Negative results	—	9
		12

Waterberg. ..		
East Coast fever, piroplasma parvum	Cattle	5
Marginal points, a probable sequel of ordinary redwater	,,	1
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	,,	2
Poikilocytosis, a probable sequel of ordinary redwater ..	,,	2
Piroplasma canis	Dogs	1
Negative results	—	42
		53
Zoutpansberg.		
East Coast fever, piroplasma parvum	Cattle	47
Ordinary redwater, piroplasma bigeminum	,,	1
East Coast fever and ordinary redwater, piroplasma parvum and p. bigeminum	,,	2
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	,,	1
Poikilocytosis, a probable sequel of ordinary redwater ..	,,	8
Acariasis, sarcoptes of Africander goats	Goats	80
Acariasis, psoroptes of sheep	Sheep	10
Anæmia, basic cells,? sequel blue tongue	,,	1
Black quarter	Cattle	2
Broncho-alveolitis	Mule	1
Cysticercus tenuicollis	Sheep	1
Piroplasma equi	Equines	9
Pneumonia	,,	1
Negative result	—	158
		322
Swaziland.		
East Coast fever, piroplasma parvum	Cattle	10
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	,,	1
Poikilocytosis, a probable sequel of ordinary redwater	,,	1
Poikilocytosis, a sequel of piroplasma equi	Equines	1
Negative results	—	17
		30
Orange River Colony.		
Piroplasma mutans	Cattle	14
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	,,	2
Negative results	—	11
		27
Rhodesia.		
Marginal points, a probable sequel of ordinary redwater	Cattle	1
Negative results	—	11
		12
Portuguese East Africa.		
Parasitic nodules	Oxen	4
Negative results	—	1
		5

Monthly Summary.

July, 1906.

East Coast fever, piroplasma parvum	Cattle	8
Marginal points, a probable sequel of ordinary redwater	„	1
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	„	3
Poikilocytosis, a probable sequel of ordinary redwater	„	1
Acariasis, sarcoptes of Africander goats	Goats	5
Acariasis, psoroptes of sheep	Sheep	3
Anthrax	Cattle	2
Diphtheria	Fowls	1
Epizootic lymphangitis, saccharomyces farciminosus	Equines	5
Glanders	„	3
Poikilocytosis, a probable sequel of piroplasma equi	„	1
Streptococci infection	„	1
Streptothrix	Cow	1
Swine fever	Pigs	1
Traumatic pericarditis	Cattle	1
Negative result	—	65
		102

August, 1906.

East Coast fever, piroplasma parvum	Cattle	15
Acariasis, sarcoptes of Africander goats	Goats	9
Acariasis, psorpotes of sheep	Sheep	1
Abscess due fo coccus infection	Equines	1
Anæmia. basic cells,? sequel of blue fongue	Sheep	1
Anthrax	Cattle	1
Ascaris swillæ	Pigs	1
Necrosis of liver	Cow	1
Black quarter	Cattle	1
Broncho-pneumonia	„	2
Cysticercus tenuicollis	Sheep	1
Epizootic lymphangitis, saccharomyces farciminosus	Equines	8
Glanders	„	6
Piroplasma equi	„	7
Streptococci infection	„	2
Traumatic pericarditis	Cattle	1
Tuberculosis	„	1
Vaginitis	Cow	1
Negative results	—	51
		111

September, 1906.

East Coast fever, piroplasma parvum	Cattle	8
Poikilocytosis, a probable sequel of ordinary redwater	„	1
Acariasis, sarcoptes of Africander goats	Goats	7
Acariasis, psoroptes of sheep	Sheep	9
Anthrax	Cattle	3
Black quarter	„	2
Broncho-pneumonia	Pigs	1
Enteritis	Fowls	1
Carried forward		32

Brought forward		32
Epizootic lymphangitis, saccharomyces farciminosus ..	Equines	2
Parasitic pneumonia	Goats	1
Parasitic pneumonia	Cattle	1
Septic metritis	,,	1
Swine fever	Pigs	1
Tuberculosis	Cattle	2
Streptothrix	Mule	1
Negative results	—	78
		119

October, 1906.

East Coast fever, piroplasma parvum	Cattle	16
Piroplasma mutans	,,	4
Ordinary redwater, piroplasma bigeminum	,,	1
East Coast fever and ordinary redwater, piroplasma parvum and p. bigeminum	,,	1
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	,,	1
Acariasis sarcoptes of Africander goats	Goats	17
Acariasis, psoroptes of sheep	Sheep	3
Angioma	Cow	1
Cysticercus tenuicollis	Sheep	1
Epizootic lymphangitis, saccharomyces farciminosus ..	Equines	2
Piroplasma canis	Dogs	1
Pneumonia	Cattle	1
Streptococci infection	Calves	1
Negative results	—	66
		116

November, 1906.

East Coast fever, piroplasma parvum	Cattle	7
Piroplasma mutans	,,	17
Ordinary redwater, piroplasma bigeminum	,,	2
Acariasis, sarcoptes of Africander goats	Goats	3
Anthrax	Cattle	1
Epizootic lymphangitis, saccharomyces farciminosus	Equines	5
Mastitis	Cow	1
Parasitic nodules	Oxen	4
Piroplasma canis	Dogs	1
Piroplasma equi	Equines	2
Septic metritis	Cattle	1
Subcutaneous emphysema	Chicken	1
Tuberculosis	Cattle	1
Stilisia hepatica	Sheep	1
Staphylococcus	Cow	1
Negative results	—	81
		129

December, 1906.

East Coast fever, piroplasma parvum	Cattle	4
Piroplasma mutans	,,	2
Ordinary redwater, piroplasma bigeminum	,,	1
Marginal points, a probable sequel of ordinary redwater	,,	2
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	,,	6
Poikilocytosis, a probable sequel of ordinary redwater	,,	1
Abscess due to bacterial infection	Calves	1
Acariasis, sarcoptes of Africander goats	Goats	2
Acariasis, psoroptes of sheep	Sheep	2
Epizootic lymphangitis, saccharomyces farciminosus	Equines	2
Leucæmia	Dog	1
Piroplasma canis	Dogs	1
Piroplasma equi	Equines	2
Coryza	Horse	1
Staphylococcus	Sheep	1
Negative results	—	86
		115

January, 1907.

East Coast fever, piroplasma parvum	Cattle	6
Doubtful East Coast fever	,,	2
Piroplasma mutans	,,	1
Ordinary redwater, piroplasma bigeminum	,,	3
East Coast fever and ordinary redwater, piroplasma parvum and p. bigeminum	,,	1
Marginal points, a probable sequel of ordinary redwater	,,	1
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	,,	4
Poikilocytosis, a probable sequel of ordinary redwater	,,	5
Abscess due to bacterial infection	Calves	1
Acariasis, sarcoptes of Africander goats	Goats	7
Amphistomum conicum	Sheep	1
Anthrax	Cattle	2
Glanders	Equines	2
Strongylus contortus	Sheep	1
Mastitis	Cow	1
Negative results	—	106
		144

February, 1907.

East Coast fever, piroplasma parvum	Cattle	12
Ordinary redwater, piroplasma bigeminum	,,	6
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	,,	8
Poikilocytosis, a probable sequel of ordinary redwater	,,	6
Acariasis, sarcoptes of Africander goats	Goats	6
Anthrax	Cattle	2
Enteritis	,,	1
Epizootic lymphangitis, saccharomyces farciminosus	Equines	2
Glanders	,,	4
Carried forward		47

	Brought forward		47
Piroplasma canis	Dogs		1
Piroplasma equi	Equines		3
Pleuro-pneumonia	Cattle		2
Tuberculosis	,,		1
Negative results	—		115
			169

March, 1907.

East Coast fever, piroplasma parvum	Cattle	5
Ordinary redwater, piroplasma bigeminum	,,	3
Marginal points, a probable sequel of ordinary redwater	,,	1
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	,,	9
Poikilocytosis, a probable sequel of ordinary redwater ..	,,	15
Abscess due to bacterial infection	Calves	1
Acariasis, sarcoptes of Africander goats	Goats	1
Acariasis, psoroptes of sheep	Sheep	3
Anthrax	Cattle	1
Black quarter	,,	2
Broncho-pneumonia	,,	1
Epizootic lymphangitis, saccharomyces farciminosus ..	Equines	3
Glanders	,,	1
Parasitic nodules	Horse	1
Piroplasma equi	Equines	2
Pleuritis	Horse	1
Pneumonia	Equines	1
Negative results	—	144
		195

April, 1907.

East Coast fever, piroplasma parvum	Cattle	9
Piroplasma mutans	,,	3
Ordinary redwater	,,	4
East Coast fever and ordinary redwater, piroplasma parvum and p. bigeminum	,,	3
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	,,	2
Poikilocytosis, a probable sequel of ordinary redwater ..	,,	4
Abscess due to bacterial infection	Calves	3
Acariasis, sarcoptes of Africander goats	Goats	6
Acariasis, psoroptes of sheep	Sheep	6
Epizootic lymphangitis, saccharomyces farciminosus ..	Equines	1
Cysticercus cellulosoe	Pigs	1
Oesophagostoma columbianum	Sheep	1
Pleuritis necrotica	Sheep	1
Pleuro-pneumonia	Cattle	2
Strangles (streptococci equi)	Equines	1
Streptococci infection	,,	1
Traumatic pericarditis	Cattle	1
Pleuritis	,,	1
Negative results	—	82
		132

May, 1907.

East Coast fever, piroplasma parvum	Cattle	12
Ordinary redwater, piroplasma bigeminum	„	5
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	„	4
Poikilocytosis, a probable sequel of ordinary redwater	„	3
Acariasis, sarcoptes of Africander goats	Goats	8
Acariasis, psoroptes of sheep	Sheep	14
Anthrax	Cattle	1
Enteritis and gastritis	Sheep	1
Epizootic lymphangitis, saccharomyces farciminosus	Equines	3
Pleuro-pneumonia	Cattle	1
Streptococci infection	Equines	1
Negative results	—	67
		120

June, 1907.

East Coast fever, piroplasma parvum	Cattle	31
Doubtful East Coast fever	„	1
Piroplasma mutans	„	3
Ordinary redwater, piroplasma bigeminum	„	5
Marginal points, a probable sequel of ordinary redwater	„	2
Basic, nucleated and polychromatic cells, a probable sequel of ordinary redwater	„	3
Poikilocytosis, a probable sequel of ordinary redwater	„	11
Acariasis, sarcoptes of Africander goats	Goats	36
Acariasis, psoroptes of sheep	Sheep	7
Acariasis	Equines	2
Broncho-alveolitis	Mule	1
Traumatic hæmorrhage	Kidney of Chicken	1
Negative results	—	42
		145

Calf Vaccine Lymph.

During the year 494,928 tubes of calf vaccine lymph were issued—the details of which appear in the following statement—representing a revenue of £4,125 4s. 8d.

1906–1907.	Transvaal.	Cape Colony.	Natal.	Orange River Colony.	Portuguese Territory.	Rhodesia.	Total.
	Tubes.	Tubes.	Tubes.	Tubes.	Tubes.	Tubes.	Tubes.
July ..	15,115	24,000	200	1,000	6,000	60	46,315
August ..	16,047	18,000	800	1,000	6,000	30	41,907
September	17,534	30,000	2,000	3,000	6,000	..	58,564
October ..	10,936	6,000	2,000	6,000	6,000	..	30,936
November ..	11,871	12,000	2,500	2,500	6,032	..	34,903
December ..	12,558	18,000	2,000	1,000	6,100	10	39,668
January ..	11,496	12,000	500	1,000	6,500	..	31,496
February ..	13,831	12,000	..	1,000	6,200	..	33,031
March ..	13,674	12,000	20,000	1,500	6,400	25	53,599
April ..	12,367	6,000	20,500	1,000	6,600	10	46,477
May ..	13,115	6,000	20,600	1,000	6,500	..	47,221
June ..	13,061	9,250	1,500	1,000	6,000	6	30,811
Total	161,605	165,250	72,600	21,000	74,332	141	494,928

I also append a statement shewing the results obtained from our calf vaccine lymph, and as will be seen the successful vaccinations amount to 94 per cent.

It is a matter for regret that of the 161,605 tubes issued in the Transvaal during the year, the returns only account for 3,590 vaccinations; in other words, it is impossible to control the efficacy of 98 per cent. of the vaccine lymph issued.

Analysis from reports received on results of vaccinations with lymph issued during the financial year ending 30th June, 1907 :—

Number of Calf.	Primary Vaccinations.				Secondary Vaccinations.			
	Number performed.	Number subsequently inspected.	Number found to be successful.	Percentage of successful vaccinations to number inspected.	Number performed.	Number subsequently inspected.	Number found to be successful.	Percentage of successful vaccinations to number inspected.
				%				%
159	159	53	43	81	33	10	5	50
160	60	14	13	91	1	1	1	100
166	119	35	35	100	163	60	60	100
168	3	3	3	100	..	..	..	..
169	28	14	14	100	..	..	..	..
172	80	37	37	100	121	74	72	97
174	64	24	22	97	415	106	54	51
175	72	32	30	91	330	100	55	55
176	13	3	3	100	29	11	11	100
179	3	3	3	100	3	3	1	33
182	94	50	48	96	452	250	135	51
189	65	50	48	96	260	130	67	55
190	54	26	26	100	159	64	26	40
192	94	52	47	90	121	17	17	100
194	30	5	5	100	38	3	3	100
197	89	13	11	77	127	16	16	100
201	8	8	8	100	..	..	..	..
204	53	7	7	100	78	12	12	100
205	55	7	7	100	117	11	11	100
Total	1,143	436	410	94	2,447	868	546	62

Horse-Sickness Serum.

The total issue of horse-sickness serum for the year, in the Transvaal, Natal, Rhodesia, Cape Colony, Orange River Colony, and Portuguese Territory amounted to 2,937 litres.

Quarter Evil Vaccine.

1,250 Double doses of quarter evil vaccine (sponsziekte) were issued from July, 1906, to June, 1907, representing a revenue of £15 12s. 6d.

Blue Tongue Vaccine and Serum.

38,937 Doses of blue tongue vaccine, of which 6,850 were sent to Natal and 1,000 to the Orange River Colony, were issued during the year; and 1,437 doses of blue tongue serum were despatched for use in the Transvaal.

Publications and Lectures.

In collaboration with Mr. Gray, the Principal Veterinary Surgeon, a series of articles were published in the *Transvaal Agricultural Journal*, under the title "Veterinary Hygienic Principles applicable to Stock in South Africa," and dealing with practically all the diseases encountered by the Transvaal farmer.

At intervals during the year I forwarded articles to various English and Continental journals, detailing experiments carried out at the Laboratory.

On the 13th July, 1906, I read a paper before the Transvaal Agricultural Union, on the subjects of horse-sickness and biliary fever which was published in the minutes of the meeting.

Visits.

On the 4th of September, 1906, I proceeded to Pietersburg in connection with horse-sickness investigations, which resulted in the introduction of the virus known as "Tzaneen." I may also mention that it was on this occasion that for the first time I had convincing proof of the fact that a mule "salted" against horse-sickness might subsequently break down in immunity and die of this disease.

An Inter-Colonial Bacteriological Research Conference was held at Capetown in November, 1906, at which I was present as a representative of the Transvaal.

In December, in company with Dr. Knuth, of Berlin, I visited Natal for the purpose of collecting ticks infected with East Coast fever, and I would like to express my best thanks to Mr. Woollatt, the Principal Veterinary Surgeon of Natal, and to Mr. Amos, District Veterinary Surgeon at Durban, for kindly forwarding me further supplies.

In connection with the proposed Gouw-ziekte experiment I visited Ermelo and district, together with Mr. Walker, the Government Veterinary Surgeon at Ermelo, for the purpose of selecting farms suitable for the required purpose.

Visits Paid to the Laboratory by Officials of other States and Colonies.

Mr. H. G. Simpson, F.R.C.V.S., stayed at the Laboratory from September to December, 1906, previous to taking up the appointment of Government Veterinary Bacteriologist to British East Africa, in order to make himself acquainted with the technique of our methods of investigation. This was the outcome of an arrangement made between Mr. Stewart Stockman, Principal Veterinary Surgeon, London, and the Colonial Office, and I hope this will lead to our establishment becoming the training school for veterinary surgeons who wish to enter the Colonial Service.

In December, 1906, Dr. Knuth, of Berlin, visited the Laboratory and passed some time here. Dr. Knuth was sent by the German Government on a journey through the various African colonies, in order to become acquainted with the conditions of these colonies from a hygienic point of view, and to investigate into the prevalence of the various established diseases; he will probably occupy the chair for Tropical Veterinary Medicine and Hygiene, which will be established in Berlin, for the purpose of affording the necessary training to such veterinary surgeons as wish to enter into their Colonial Service. I was able to afford Dr. Knuth every facility regarding our work, of which he took full advantage.

Mr. Chase, M.R.C.V.S., of Bechuanaland Protectorate, Mr. Power, M.R.C.V.S., of Natal, Mr. Pereira, Veterinary Surgeon of Portuguese East Africa, and Dr. Amaral Leal, of Lourenco Marques, all visited the Laboratory at various dates, for the purpose of being instructed in our method of inoculating mules against horse-sickness.

Increases and Alterations in Staff.

During the past fiscal year, the services of three lay officials were dispensed with, one being transferred to another Division. In addition, Mr. Cameron resigned on the 1st July, 1906.

The institution of a branch to deal with the vaccination against rabies, necessitated the appointment of a new official, and Dr. Walter Frei was engaged on the 21st November, 1906, as Assistant Government Veterinary Bacteriologist. Dr. Frei is a graduate of the Veterinary Faculty of the University of Zurich, where he was assistant to Professor Zangger, and acted, *ad interim*, as Lecturer of Anatomy. Later he was Assistant at the Physiological Laboratory at the Poppelsdorf Agricultural Academy, near Bonn, Germany. Dr. Frei commenced his duties at the beginning of January at the Pasteur Institute of the Province de Brabant in Brussels, under the tuition of Professor Bordet, where he passed three months in acquiring the necessary knowledge and technique required for the preparation of rabies vaccine, and treatment. Dr. Bordet, whose name is well known in connection with the preventive inoculation against rinderpest, was good enough to render Dr. Frei every assistance, and accordingly our best thanks are due to Dr. Bordet. Dr. Frei is also acquainted with the modern chemico-physical methods of investigation, and with serology, his thesis on Hæmolysis affording the best proof of this fact.

The increase in connection with the administrative and technical work necessitated the appointment of a technical clerk, and Mr. H. W. R. King was transferred from another department, and commenced his duties on the 15th August, 1906. The work connected with this post chiefly consists in attending to all correspondence of a technical nature, and in assisting me in compiling reports, etc.

Mr. J. H. F. Cox was appointed as clerk in the Administrative Branch, in July, 1906.

Mr. T. Meyer commenced duty on the 1st July, 1906, as Laboratory Assistant.

Mr. F. T. Mauchle filled the remaining vacancy, and was appointed on the 15th June, 1907.

Leave of Absence.

In February, 1907, Mr. Dodd contracted enteric, but recovered, and was still on sick leave at the end of the fiscal year.

Mr. E. Heron was granted six months vacation leave, from the 6th June to the 6th December, 1906.

Administration.

I append herewith report furnished by Mr. E. B. H. Parkes, Superintendent, relative to the work under his direct control:—

To the Government Veterinary Bacteriologist.

I have the honour to present for your information and reference, and for the information of the Director of Agriculture, a review of the general work of the administration of this Division during the past year.

The sum of money voted by the Legislative Assembly for the current expenditure of the year was £30,759 and the total expenditure amounted to £30,964. Of this sum £5,752 was expended in salaries and £2,236 in native wages; the remainder was spent as under:—

Purchase of live stock	£10,983
Maintenance of live stock	5,700
Compensation for mules dying under inoculation	2,202
Purchase of laboratory apparatus, general stores and equipment and sundries ..	3,815
Rent and insurance	276

It will be seen that by far the heaviest expenditure in connection with this Division is in the cost of animals for experiment and on their maintenance, and it was realised in December, 1905, that the unsuitability of the site of the Laboratories at Daspoort as a place to keep large numbers of animals militated very seriously against all endeavours to keep the latter expenditure within bounds. To partially remedy this state of things, the farm Linwood was leased at that time, and later the farm Sjamboks Oude Kraal was hired for winter grazing, but although by this means an economy in the maintenance of live stock was effected, the remedy could only be said to be partial, as in both cases the farms were situated at a distance from the Laboratories, and consequently only such animals as were not immediately required in the Laboratories could be sent to them, and in neither case was there any convenience for the protection of live stock, nor was it possible to grow crops to supplement the grazing for such animals as were in need of extra care and feeding. The rapid increase in the work at the Laboratories, and in consequence the increase in the number of animals required for experiments, made it clear that the inadequacy and unsuitability of both the laboratory and stable accommodation necessitated that the whole question of the future of this Division should receive careful consideration, and the outcome of this was the purchase in November, last year, of a portion of the farm Onderstepoort as a site for a new laboratory, and the commencement of the new Laboratory buildings which are now under construction.

The number of animals purchased during the year were:—

362 horses, 284 mules, 40 donkeys, 60 cattle, 300 sheep.

As all of these animals were purchased for experimental purposes, and generally some special qualification was necessary, it was not possible to obtain them as cheap as might have been done were all sources of supply available. To explain what I mean, it was necessary that the sheep should have been bred and lived in a district in which blue tongue was unknown, and for this reason they were imported from the Cape Colony. The cattle had to be brought from a district free from South African redwater, and it was necessary that the donkeys and mules should have been recently imported from the Argentine to eliminate the possibility of their having previously contracted and recovered from either horse-sickness or biliary fever. The horses alone, with the exception of a few from the Argentine, which were procured for a special experiment, could be selected without regard to their origin and previous history, and in purchasing these, as a rule, the cheapest horses obtainable have been bought. Of the 362 horses purchased, 190 were obtained at prices ranging from £5 to £10 each, but for the remaining 172 larger prices had to be paid.

There has been a constant difficulty in obtaining a sufficient number of horses for experiment at a reasonable price, but although the large demand for the class of animal required has undoubtedly increased their market value

in Pretoria, the prices paid compare very favourably with prices paid in the past.

The number of horses that have died under experiment is large, and up to now it has not been advisable to buy any but the cheapest that can be obtained; but with mules, however, it is different, as the greater number of those that died were purchased at a very cheap rate for virus, and were expected to die, and of the others the losses chiefly occurred at the early part of the year in experimenting to fix the dose of serum for general use, and the death rate amongst the mules which were used to test the serum was not unduly greater than the death rate through inoculation all over the country. For the purpose of testing serum consequently, it was thought desirable to purchase a class of mule that would be readily saleable again, and accordingly young sound mules were purchased at prices ranging from £17 to £20 each. Before proceeding to the cost of the upkeep of live stock, it will be well to consider the number of the animals kept and the way these were fed.

During the year the average daily number of animals kept at Daspoort has been—

139 horses, 60 mules, 15 donkeys, 46 cattle, 124 sheep and goats.

In addition to these, a number of animals were out at grass at Linwood, Onderstepoort, and at Sjamboks Oude Kraal; the daily average at grass, taking these three farms together, was—

48 horses, 154 mules, 18 donkeys, 53 cattle, 200 sheep and goats,

so that the daily average number of animals kept by the Division was—

187 horses, 214 mules, 32 donkeys, 99 cattle, 324 sheep and goats.

In the feeding of live stock so large a sum of money is expended that it is a matter to which particular attention has been paid, as it seemed that if a more economical manner of feeding could be adopted, considerable economy might be effected, and, in fact, this has proved to be the case. The appointment of a yard foreman at the commencement of the year has facilitated this, and has also resulted in a very noticeable improvement in the condition of all the live stock on the station, and although a certain number of deaths from natural causes must always occur where a large number of animals of such a poor class are kept, I believe that there has been a marked decrease in mortality due to these causes.

In the feeding of serum horses, it has been ascertained that it is a better policy to keep them always in the highest condition, since the cost of the extra feeding is recompensed by it being possible to obtain larger quantities of serum, and consequently it is not necessary to keep so large a number of animals for the purpose. Previously it was the custom to bleed serum horses once a month and to take at each bleeding four litres of blood, but it was found that a large number could not be bled as often even as that, and that the average number of days' interval at which each horse was bled was nearly forty days. With better feeding, it has now been found possible to bleed all horses every fourteen days, and horses which do not keep their condition under this treatment are rejected, and so the cost of their feeding is saved. By keeping a smaller number of horses to produce the same quantity of serum, even though the actual cost per head is somewhat higher, the cost per dose of serum has been very materially reduced, and in this manner there is some prospect of it being possible in the near future to reduce in like proportion the fees charged for inoculation.

It has been also found a sound policy to keep all live stock that are under experiment in as good condition as possible, so that when they are discharged a heavy expenditure may not be incurred in getting them into condition for sale or loss through selling when in a poor and debilitated condition.

The total sum expended on corn and forage amounted to £5,700, which was £800 less than was spent in the previous year, although the number of animals kept had certainly in the latter months of the year largely increased.

The quantities of corn and forage used were—

125 tons oats, 250 tons mealies, 50 tons bran, 27 tons oil-cake, 6 tons salt, 215 tons oat forage, 210 tons veld hay, and 50 tons green forage, and in addition about 200 tons of green forage that was grown at Onderstepoort.

The animals at Daspoort have, of course, had to be entirely fed on purchased corn and forage, with the exception of the relatively small quantity of green forage that was sent in from the farm in the latter months of the year. In addition to these animals at Daspoort, the horses that were out at grass received a ration of corn all the year round, as these were the horses that were used for the preparation of serum. The mules also during a part of the year had to receive some additional food, owing partly to the scarcity of grass and partly to a number of them being in a poor condition when they were sent out to the farm. It is probable that had all the mules been in good condition in the autumn that they would have come through the winter well on the grazing at Sjamboks Onde Kraal. This, however, was not the case, as the majority of them were sent out to the farm just after recovering from either horse-sickness or biliary fever or both, and were in a low condition from the commencement.

With such a large number of animals, it is not surprising that the cost of feeding them is a very heavy item, and it is made the more so through the necessity of having to keep so large a number of them at the Laboratory at Daspoort, where, of necessity, they had to be kept stabled and fed on purchased forage all the year round.

On account of the want of accommodation, it has always been necessary to keep a much larger number of animals at Daspoort than would be necessary had there been stables on the farm. Horses and other animals after having passed through an experiment, and which will subsequently be used again in further experiments, have to undergo a period of rest during which they may eliminate from their systems any results that might remain from the previous treatment, and during this time it is, of course, necessary that they should be under observation, and that their temperatures should be taken twice daily. These animals could all be out at grass during the day if there was accommodation for them to be stabled at night, but from the lack of this accommodation it has been necessary to keep them at Daspoort, whereby the cost of their feeding is enormously enhanced. Again, animals in the early stages of experiment could always with advantage be allowed to graze during the day; and, in fact, it is reasonable to suppose that the more normal the condition under which they are kept and the more those conditions assimilate to such as would occur in practice, the more reliable will be the deductions from the experiments. From what I have said it will be seen that the ideal state would be an abundance of grazing of good quality and in close proximity to the Laboratory, as it would not be possible to send these animals long distances twice a day to their grazing. To comply with these conditions for such a large number of animals a farm would be necessary with pasture such as is not to be found in South Africa. The best alternative, however, which is possible, is a partial soiling system, so that all animals may have their daily run at grass and the deficiency in feed made up by supplies of green forage. The advantage of this system also would be that the farm will be continually increasing in fertility, so that in time it will be able to carry three or four times the head of stock that it would do at present, and so more nearly conform to the ideal desired.

This was one of the chief reasons for advocating that the new Laboratory should be situated on a farm where all live stock, when not too sick, can have a daily run at grass and in addition be supplied at all times with ample quantities of green forage.

This latter is most necessary for animals kept under the abnormal conditions such as must maintain in an experimental station; for, on consideration, it is clear that the horses that are used for the preparation of serum naturally do not require to be fed as if they were horses in hard work; on the contrary, although high feeding is absolutely necessary to help them to withstand the strain of constant bleeding, soft and easily digested food is indicated, and under a liberal allowance of green forage these horses have been found to keep better condition and stand the constant bleeding better than on dry food alone. A trial was also made with cooked food, and although from want of convenience for continuing this on a large scale the experiment could not be fully carried out, it was sufficient to show that it is a matter which is worth going into more fully when it is possible with the better accommodation at the new Laboratory. The horses under experiment again must be considered as sick animals, and consequently require careful feeding, for which purpose green forage is most suitable, and when convalescent they need to be fed up into condition again on a liberal diet. It must also be borne in mind that, as all horses are in the first instance purchased with a view to experiment, and that only a small proportion eventually have up to now survived all experiments, that only the cheapest horses obtainable are bought, and it is even difficult sometimes to get horses at all at a reasonable price; consequently it is not surprising that a certain number of them are old, in poor condition, and broken down in constitution through hard work, and these do not always quickly respond even to liberal feeding. It is necessary, therefore, sometimes to keep them for a long time before they are fit to be bled, which up to now has been the ultimate destiny of all horses that, after passing through all tests, have become salted.

The remarks that I have made with regard to horses apply in an equal degree both to cattle and sheep. Cattle are all used first for the production of vaccine lymph, and during the time that they are under treatment for this purpose a very severe strain is put on the animals; as soon as they have recovered from this they are then used for experiment in redwater and other diseases, which is again a heavy tax on the animals' constitution. During the whole time that these cattle are kept at Daspoort it is necessary therefore that they should be very highly fed, and there is no doubt that the best possible food for them would be green forage, supplemented by an allowance of corn and oil-cake when such is found necessary.

Sheep were and will be more largely kept in future for the preparation of vaccine against blue tongue, and in their case. again, they not only have to undergo the disease, but are also bled to obtain the blood from which the vaccine is prepared. It was not anticipated last season that these sheep, since they are only bled once, would require much extra feeding, and as soon as they had returned to a normal condition of health after bleeding they were sent out to grass. The result, however, was disastrous, as not only did they at once fall away in condition, but a large percentage died, and that after they had been put back on to a liberal allowance of corn. It is probable that had they been fed from the beginning on green lucerne, this would not have occurred, and that in future with an abundant supply from the farm that the whole of the sheep may be saved and the cost of feeding not be excessive, and it would, of course, be impossible to produce blue tongue vaccine at a penny a dose if heavy expenditure on feeding, in addition to a large loss through deaths, were to continue. All other animals that are under

experiment have for similar reasons to be well looked after and well fed, and it is obviously not possible to make a comparison in the cost of feeding animals kept under such conditions as they must necessarily be at these Laboratories with the cost of feeding animals kept under normal conditions.

From what I have said it will be clear that for all animals on this station the most suitable feed is green forage. Unfortunately up to now the cost of this has made it practically impossible to use it in any quantity.

The market price of green forage varies from as much as 7s. per 100 lbs. to 2s. per 100 lbs., and may be said to be rarely lower than the latter price, except for short intervals when there is a glut in the market. The average price paid during the year has been about 4s., and at that price it is plain that the expense of feeding it in large quantities would be altogether prohibitive, and in consequence, unless green forage can be grown on the place, that the most suitable manner of feeding animals is out of the question.

It was late in the season before a farm was purchased for a site for the new Laboratory, and it was not until the 14th of November that a commencement could be made with cultivation. The portion of the farm Onderstepoort purchased, which is situated about two miles north of Wonderboom Station on the Pretoria–Pietersburg line, is 237 morgen in extent, and has the Aapies River for its eastern boundary, with the farm Wonderboom to the south. The Pietersburg Railway passes through the farm, as also the main road to Pienaars River. There are about 80 acres which are capable of being put under irrigation, but of this not more than 10 acres were previously under cultivation. The whole 80 acres have now been ploughed up, and some dry land in addition, but all of this could not be put under summer crops last year, and it was not to be expected that the results during the first season would be good. The lateness in the year prevented the thorough cultivation necessary for preparing new lands for the summer crops, and considerable damage was done by locusts, but something like 200 tons of green forage was cut and fed to the animals with a very marked good effect on their condition and health. The prospects for the winter crops are, however, extremely good, and there are about 53 acres under oats and rye and 20 acres under lucerne. A larger area could not be put down in lucerne owing to the lateness of the season at which farming operations were begun and to the state of the land being such that until thorough cultivation had been given it would have been useless to attempt to lay down lucerne. It is, however, intended to sow the whole of suitable land with this crop, and during the coming season another 25 or 30 acres will be sown.

The work that is carried on in these Laboratories cannot be immediately reproductive in a commercial sense, and this is only to be expected, for the benefits to the country at large of new discoveries are not things that can be reckoned in pounds, shillings, and pence; nevertheless, preparations for the prevention of disease are sold and a certain revenue is obtained. The price fixed for these preparations is made as low as possible, and, of course, does not actually in many instances cover the cost of production, for in estimating this, in order to fix a price, that part of the first initial cost which may be considered as the ordinary routine work of the Laboratory is not taken into account. The aim has been rather to cover the extra cost that would be thrown on the Division by the extra expenditure that would be incurred to increase the output where the demand for any Laboratory products largely increased. In addition to the various Laboratory products sold, the fees for the immunisation of mules amounted to a large sum, and there was a considerable revenue from the sale of animals which were no longer required for experiments. The revenue from the sale of live stock was £6,006; 102 mules were sold out of hand at an average price of £22 4s.,

and 136 mules were sold by auction and realised an average of £16 11s. 6d. In addition to these a number of mules were supplied to other Divisions of the Department and some to other Government Departments—in all to the value of over £1,000. A few cattle and donkeys were also sold.

Horse-sickness serum was sold to other Colonies and Foreign Governments to the value of £1,825, and the fees for the inoculation of mules realised £4,924. From this latter, however, must be deducted the amount of £2,202 paid out in compensation to those owners of mules whose animals died whilst undergoing the treatment, so that the nett proceeds only amounted to £2,722.

The fees for the immunisation of mules it may be possible to reduce in the future, although this has in the past probably barely covered the cost of the preparation of serum and of the expenses incidental to inoculation, together with the cost of compensation for mules dying under inoculation. Economies are possible in two ways, first, the cost of production may be reduced; second, the percentage of losses under inoculation may be reduced and so the cost of compensation be lessened. The latter, I understand, there is good prospect of effecting. The former has been materially reduced by obtaining a larger quantity of serum from each horse, as I have explained above, and also, now that the preparation is no longer in an experimental stage, by making it in larger quantities and testing it in bulk, by which a smaller number of mules are required to be provided for this purpose, and the expenditure on their upkeep is saved. The revenue from vaccine lymph satisfactorily covers the cost of production, although the price charged is far less than lymph can be obtained for in most other countries. No revenue was obtained from the sale of blue tongue vaccine, as, being in an experimental stage, it was issued last year free of cost, but in the present year a charge of 1d. per dose will be made, which, it is anticipated, will cover the cost of production if there is a demand for large quantities.

The total revenue received was as under:—

Sale of live stock	£6,006
Fees for inoculation of mules	4,924
Sale of horse-sickness serum	1,825
Sale of vaccine lymph	4,125
Sale of other Laboratory products and sundries ..	233
Total	£17,113

The gross expenditure was £30,964, so that the nett expenditure, after deducting the revenue, only amounted to £13,851.

In the years 1905-6 the nett expenditure was £20,037, or over £3,000 more than during the year that is past.

I have the honour to be,
Sir,
Your obedient Servant,
E. B. H. PARKES,
Superintendent.

* * * *

Interviews and Correspondence.

Numerous interviews were accorded to farmers and others interested in stock diseases and also in the work of this Laboratory.

As will be seen from the following statement, a remarkable increase occurred in the correspondence, and I am glad to record that farmers seem to recognise the utility of our Division, frequently enquiring on stock diseases, and in many cases forwarding specimens for examination.

RETURN OF LETTERS AND TELEGRAMS RECEIVED AND DESPATCHED DURING THE FINANCIAL YEAR ENDING THE 30TH JUNE, 1907, AS COMPARED WITH THE CORRESPONDING PERIOD FOR THE PREVIOUS YEAR.

	1906–1907.						1905–1906.		INCREASE IN 1906–1907.	
	LETTERS.		TELEGRAMS		TOTAL.		TOTAL.		TOTAL.	
	Received.	Despatched.	Received.	Despatched.	Received.	Despatched.	Received.	Despatched.	Received.	Despatched.
Administrative Office	1,289	781	Included in Letters.	309	1,289	1,090	2,517	2,837	1,155	939
Technical Office ..	2,383	1,845		841	2,383	2,686				
	3,672	2,626		1,150	3,672	3,776	2,517	2,837	1,155	939

THE NEW BACTERIOLOGICAL LABORATORY AT ONDERSTEPOORT.

In continuation of my last annual report regarding the prevalency of enteric fever at the Laboratory, I am glad to say that the late Government decided to remove our quarters to a fresh site, and the present Parliament carried on the scheme, voting an additional sum for the purpose. We were able to purchase a suitable site in the neighbourhood, on the farm "Onderstepoort." This farm offers suitable conditions for our purpose, as it is within easy reach of Pretoria; a railway line passes quite close and a siding will be erected; the Aapies River forms one of the boundaries, and sufficient land can be irrigated for grazing and cultivation of our own crops.

The plans were designed by Mr. Eagle, Chief Architect of the Public Works Department; those of the main buildings, including the Laboratories and Administration Offices, have been approved, and these buildings were commenced at the close of the fiscal year.

In conclusion, I wish to place on record the good work performed by the staff, especially during the summer months.

I have the honour to be,

Sir,

Your Obedient Servant,

A. THEILER,

Government Veterinary Bacteriologist.

"A."—FURTHER NOTES ON PIROPLASMA MUTANS — A NEW SPECIES OF PIROPLASMA IN SOUTH AFRICAN CATTLE.

In my last Annual Report I established the fact that the rings and rods which sometimes appear in susceptible cattle after the injection of redwater blood have no connection with redwater (P. bigeminum), but must be considered as a separate piroplasm, which I have designated Piroplasma mutans.

The chief argument in favour of this is that an animal can be infected with redwater exclusively, and at any later period with piroplasma mutans. The incubation time of this latter parasite varies from 20 to 45 days, whereas the incubation time of redwater is from five days onwards. Naturally piroplasma mutans appears after the disappearance of piroplasma bigeminum.

I have in addition shown that not all the animals in the Transvaal are infected with piroplasma mutans, but those which are susceptible can easily be infected when injected with blood containing this new piroplasm.

The experiments I now bring forward will (1) add further proof of the duality of the two piroplasms, (2) show that the blue tick, which is the carrier of piroplasma bigeminum, does not transmit piroplasma mutans, (3) show that piroplasma mutans is distributed all over South Africa, and (4) show that the injection of blood containing piroplasma bigeminum and piroplasma mutans into cattle susceptible to ordinary redwater does not always cause the former parasite to appear.

Experiment No. 1.

Infection of animals known to be immune against redwater with piroplasma mutans.

The animals belonging to this experiment were originally considered as susceptible to redwater, but on examination of their blood it was found to contain piroplasma bigeminum, and therefore they were immune against this disease.

"*A*," *Calf* 382.—About one year old; was injected subcutaneously on the 11th June, 1906, with 10 c.c. blood of calf 359.

No reaction appeared consequent on the inoculation, and since in the meantime it was found that 382 was immune against redwater (piroplasma bigeminum) it was injected subcutaneously on the 6th July—25 days after the first inoculation—with 10 c.c. blood of heifer 316, an animal which contained both piroplasma bigeminum and piroplasma mutans in its blood. Nothing particular happened after this injection until the 36th day—11th August, 1906—when rings and rods were noticed, and a slight disturbance of the temperature ensued. These rings and rods increased slightly during the following days, but they were never present in great numbers. The lesions of poikilocytosis were noted on one occasion during their presence—viz., on the 20th August, 1906. Rings and rods were seen on the 11th and 12th September, and again on the 20th October, 1906—36 days after the second injection. The examinations were discontinued on the 20th October, 1906.

"*B*," *Calf* 384.—Injected on the 11th June, 1906, with 10 c.c. blood of calf 359 (*vide* Experiment No. 1, "*A*").

Nothing happened with this heifer for the same reason as in the former case. It was therefore decided on the 26th day—6th July—to inject 384 with 10 c.c. blood of heifer 316, containing both piroplasma bigeminum and piroplasma mutans. This inoculation produced a distinct reaction, during which piroplasma bigeminum was noticed on the 10th day, and again on the 14th day after the second injection. The reaction subsequently subsided, but from the 35th day another rise of temperature ensued, and three days later—13th August—rings and rods were noticed for the first time. These rings and rods increased, and during the time they were most frequent a febrile reaction was present; the lesions of poikilocytosis were also very pronounced. Rings and rods gradually decreased, and finally disappeared on the 71st day—15th September, 1906—when the examinations were discontinued.

"*C*," *Heifer* 386.—Belonging to the same lot as animals 382 and 384, and was considered susceptible to redwater, but examination proved the presence of piroplasma bigeminum in its blood.

Injected subcutaneously on the 29th June, 1906, with 10 c.c. blood of Cape animal 380 known to be infected with piroplasma bigeminum. On the 12th day after inoculation a reaction ensued, but examinations of blood proved negative. Irregular temperatures were noticed for some time after this. Fifty-six days later—19th October—it was decided to inject 386 with 5 c.c. blood of heifer 316—immune to piroplasma bigeminum and piroplasma mutans. Thirty-two days later—20th November—rings and rods were present; they

increased in number, and coinciding with their increase a rise of temperature ensued; the lesions of poikilocytosis became very pronounced. Rings and rods were daily present for over five weeks, and were still noticed in rare numbers on the 72nd day after the third inoculation—31st January, 1906—when the examinations were discontinued.

"*D*," *Heifer* 404.—About two years old, directly imported from Aliwal North, and therefore susceptible to redwater.

Was injected on the 16th October, 1906, subcutaneously with 5 c.c. blood of calf 396. This calf had been previously injected with piroplasma bigeminum blood (compare Experiment No. 3, "*E*"), and at the date of injection into heifer 404 was immune against redwater. After an incubation time of 16 days the temperature of heifer 404 rose to 105 F., and four days later piroplasma bigeminum and poikilocytosis were noticed. The temperature now oscillated, and basic cells were present on one occasion. The lesions of poikilocytosis were frequently noted from the 20th to the 37th day, and afterwards at rare intervals. On the 106th day after injection of blood of 396—30th January, 1907—heifer 404 was injected subcutaneously with 10 c.c. blood of heifer 425 immune to piroplasma mutans (see Experiment 6, "*C*"). Irregular reaction noted, probably due to spirillum; all blood examinations negative. Second reaction commenced on the 24th day, recording 106 on the 43rd, 44th and 45th days. Piroplasma mutans noted during the reaction on the 29th, 32nd, 34th and 36th days. Poikilocytosis and marginal points also frequently noted until the 47th day. All further examinations negative, and were accordingly discontinued from the 72nd day.

"*E*," *Heifer* 412.—This was an Aliwal North two-year-old animal, and susceptible to redwater. Accordingly on the 16th October, 1906, it was injected subcutaneously with 5 c.c. defibrinated blood of calf 387 (compare Experiment 3, "*B*"; calf 387 was immune against redwater, and although infested with blue ticks which had previously been feeding on animals containing both piroplasma bigeminum and piroplasma mutans in their blood, calf 387 did not contract piroplasma mutans, since, as I shall prove later, the blue tick is not the carrier of this new piroplasm).

Reaction commenced on the 8th day after injection, and four days later recorded 105.4. Examinations on the 9th and 12th days gave negative results. The temperature now fell and remained between 99 and 103 from the 19th to the 82nd days. Piroplasma bigeminum noted for the first time on the 13th day after injection; spirillum present the following day. Piroplasms again noted on the 15th, 16th and 18th days. The lesions of poikilocytosis, together with basophile cells, were present on the 21st day, and two days later basic cells appeared. Poikilocytosis was occasionally noted from the 27th to 60th days, and piroplasma bigeminum was again present on the 32nd and 36th days.

On the 83rd day—January 30th, 1907—heifer 412 was injected with 10 c.c. blood of heifer 425 immune to piroplasma mutans (compare Experiment 6, "*C*"). The lesions of poikilocytosis noted on the 8th and 9th days after this injection, and again on the 13th and 15th days; a slight reaction ensued from the 15th day, recording 105.8 two days later, and regaining normal on the

23rd day. All examinations during this reaction gave negative results. Poikilocytosis was frequently noted from the 24th to the 32nd days, and five days later (37th day) a rise of temperature from 101.4 in the morning to 103.8 twelve hours later was accompanied with the presence of piroplasma mutans for the first time. Piroplasma mutans and poikilocytosis were present the following day, and the temperature dropped. Another short reaction noted from the 43rd to the 50th days, when poikilocytosis and piroplasma mutans were again present for two days.

All further examinations were negative, although the temperature showed an irregular record. The experiment was discontinued on the 13th April, 1907, 71 days after injection of 425.

Conclusions.

The injection of blood of an animal which we knew did not contain piroplasma mutans failed to produce this parasite in the injected animal, but the same animals shewed piroplasma infection directly they were injected with blood containing piroplasma mutans. These parasites appeared within the typical incubation time, viz., in the first instance, after 36 days; secondly, after 35 days; thirdly, 32 days after injection; fourthly, after 29 days; and lastly, after 37 days. The lapse of time between the inoculation of blood known not to contain piroplasma mutans and the injection of blood containing this parasite is long enough to prove that piroplasma mutans infection is not due to the first injection. This period was in the first instance 61 days; secondly, 60 days; thirdly, 88 days; fourthly, 106 days; and lastly, 83 days.

Experiment No. 2.

Heifers infected with blue larval ticks in the first instance, causing a pure infection of piroplasma bigeminum, and subsequently injected with blood containing piroplasma mutans.

"*A*," *Heifer* 398.—Aliwal North animal, and therefore susceptible to piroplasma bigeminum. Infected on the 16th October, 1906, with numerous blue tick larvæ of heifers Nos. 314 and 316—animals immune to redwater and piroplasma mutans, therefore containing both piroplasma bigeminum and piroplasma mutans in their blood.

Nothing unusual occurred until the 22nd day, when a slight rise of temperature was noticed, and piroplasma bigeminum appeared and remained for the four following days; a slight poikilocytosis also resulted from this infestation.

Sixty-four days after the tick infestation, 398 was injected with blood of 316, an animal, as stated above, containing piroplasma mutans and piroplasma bigeminum in its blood. Twenty-five days after this injection the first flagellated form was seen and poikilocytosis was noticed as a result. The parasites increased in numbers, and remained present for some time.

"*B*," *Heifer* 402.—Aliwal North animal, and therefore susceptible to piroplasma bigeminum. Infested on the 16th October, 1906, with numerous blue tick larvæ of heifers 314 and 316, animals containing both piroplasma in their blood. On the 21st day a rise of temperature was noticed, accompanied with the appearance of spirillum for five days. On the 32nd day piroplasma

bigeminum was noticed and remained for six days, followed by a slight poikilocytosis; piroplasma bigeminum was again noticed on the 43rd and 45th days. On the 64th day this animal was injected with blood of heifer 316—containing both piroplasma bigeminum and piroplasma mutans. On the 23rd day after this injection the first rod-shaped parasite was seen. It increased in numbers, causing a more distinct poikilocytosis and a slight rise of temperature. The parasites remained present for some time.

"*C*," *Heifer* 405.—Aliwal North animal, and therefore susceptible to piroplasma bigeminum. Infested on the 16th October, 1906, with numerous blue tick larvæ of heifers 314 and 316—animals immune to piroplasma bigeminum and piroplasma mutans, therefore containing both these parasites in their blood. On the 20th day after this infestation a rise of temperature was noticed, which was succeeded by the appearance of spirillum. On the 34th day after infestation another rise of temperature was observed. This curve was succeeded by the appearance of piroplasma bigeminum, which was present for the four following days. Piroplasma bigeminum was again present on the 43rd and 45th days, and the poikilocytosis continued for some time. On the 64th day after infestation, the animal was injected with blood of heifer 316, containing both piroplasma bigeminum and piroplasma mutans. Twenty days after this inoculation the first rod-shaped parasite was seen; they were noticed in rare numbers, but four days later—24th day—increased, causing a slight rise of temperature, together with a slight poikilocytosis, and the appearance of marginal points. The rings and rods remained for some days.

"*D*," *Heifer* 407.—Aliwal North animal, about two years old, and susceptible to redwater.

Infested on the 16th October, 1906, with blue tick larvæ which had previously been feeding on Madagascar ox 347, immune to piroplasma bigeminum and piroplasma mutans (see Annual Report, 1905-6, page 59, No. "A"). The temperature reached 104.2 on the 6th day, but dropped and recorded from about 99.6 in the morning to 103.4 in the evening from the 10th to the 16th days, during which time all microscopical examinations gave negative results. A sharp rise was now noted from 101.2 in the morning of the 17th day to 105.6, 36 hours later, and returning to a normal record on the 21st day, from which date another rise was recorded, the temperature touching 104 on the 22nd and 23rd days. The lesions of poikilocytosis were noted on the 21st day, followed by the daily appearance of spirillum until the 25th day.

The blue tick adults commenced to fall on the 20th day, and for the two following days poikilocytosis was present. On the 32nd day the temperature rose from 101 to 103.8 in the evening, and coinciding with this, piroplasma bigeminum was noted for the first time. The temperature now fell and touched 99 F. on the 35th day, but piroplasma bigeminum was noted daily from the 33rd day to the 37th day. An irregular temperature record was shown from the 38th to the 105th day, occasionally touching 104 F. in the evening. Piroplasma bigeminum was again present

on the 39th day after infestation, and, with the exception of the occasional appearance of poikilocytosis, no further points were noted. On the 106th day (January 30th, 1907) heifer 407 was injected with 10 c.c. blood of heifer 425 immune against piroplasma mutans (compare Experiment 6, "*C*"). Reaction from the 12th to the 20th day, recording 104.2 on the 16th day; all examinations from the 8th to the 23rd day negative. Another febrile reaction noticed from the 24th day, and on this date slight poikilocytosis and a few ring forms were noted, these lesions being again present on the 26th and 27th days. During this time the temperature was slowly rising, and on the 29th day poikilocytosis and a few flagellated forms were noted, followed two days later by the presence of piroplasma mutans, the temperature on that date being 104.2. From the 34th and 35th days, when piroplasma mutans was still present, the temperature slowly fell, recording 98.8 in the morning of the 46th day, but rose sharply to 104.4 in the evening, the same record being noted the following morning, when piroplasma mutans and poikilocytosis were noted. The temperature remained high for the next eight days, on which latter date—54th after injection—piroplasma mutans was again present. All further examinations were negative, and the experiment was discontinued from the 71st day after injection of blood from 425.

"*E*," *Heifer* 408.—About two years old; imported from Aliwal North, and therefore susceptible to redwater. Infested on the 16th October, 1906, with blue tick larvæ, which had previously been feeding on ox 347. This was a Madagascar ox (compare Annual Report, 1905-6, page 59, No. "A") immune to redwater and piroplasma mutans. An irregular temperature was noted for the first 16 days, during which time all examinations were negative. A sharp rise from 100.4 in the evening of the 18th day to 106 48 hours later was followed by the appearance of spirillum on the 21st, 23rd and 25th days, by which time the temperature had regained normal. Another sharp rise was now noted, reaching 106 on the 27th day, and on the following day the blue ticks commenced to drop. The temperature now shewed a difference of about 4 F. between the morning and evening records for the next eight days, and on the 31st day after infestation piroplasma bigeminum was noted for the first time.

These piroplasms were present daily from the 32nd to the 37th day, and from this date the temperature became very irregular, recording between 100 and 104 for the next 69 days, during which time frequent microscopical examinations were made, but with negative results. On the 106th day after infestation (30th January, 1907) heifer 408 was injected subcutaneously with 10 c.c. blood of heifer 425, immune to piroplasma mutans (compare Experiment 6, "*C*"). Reaction noted from the 23rd day; all examinations from the 8th to the 26th days gave negative results, but on the following day the lesions of poikilocytosis appeared, and were present on the 29th day, accompanied with flagellated forms in rare numbers. Piroplasma mutans noted on the 31st day, accompanied with poikilocytosis, and were both present on the 34th and 36th days. From this date an irregular temperature record was noted, lasting until the 71st day, when the experiment was discontinued. With the

exception of the presence of poikilocytosis on the 51st and 55th days, all further examinations were negative.

"*F*," *Heifer* 409.—About two and a half years old; imported from Aliwal North, and therefore susceptible to redwater.

Infested on the 16th October, 1906, with blue tick larvæ previously feeding on heifers 314 and 316, animals immune to piroplasma bigeminum and piroplasma mutans (compare Annual Report, 1905-6, pages 44 and 45). Rise of temperature from 23rd day, reaching 105.6 three days later, and accompanied with spirillum during the 24th, 25th and 26th days. Adults dropped on the 28th day, and the tempearture slowly fell, recording 100 on the 33rd day, rising to 105 on the following morning, and accompanied with piroplasma bigeminum, which was present for the next three days. An irregular temperature record was now noted until the 105th day, poikilocytosis occasionally being noted. On the 106th day—30th January, 1907—heifer 409 was injected with 10 c.c. blood of heifer 425, immune to piroplasma bigeminum and piroplasma mutans (compare Experiment 6, "*C*"). Irregular reaction followed, poikilocytosis frequently being present, and on the 22nd day rings and flagellated forms were noted in rare numbers. These rings and flagellated forms increased during the next few days, but were present in rare numbers on the 29th and 31st days. The temperature record continued to be of an irregular character, until the 72nd day, when the experiment was discontinued.

Conclusions.

All the six animals—398, 402, 405, 407, 408 and 409—were susceptible to piroplasma bigeminum; they were all successfully infected with blue ticks, which caused the appearance of piroplasma bigeminum exclusively.

Sixty-four days after the infestation of animals 398, 402 and 405, and 106 days after that of 407, 408 and 409, they were injected with blood of an animal containing both piroplasma bigeminum and piroplasma mutans. This injection caused the appearance of piroplasma mutans after the typical incubation time—398 after 25 days, 402 after 23 days, 405 after 20 days, 407 after 29 days, 408 after 29 days and 409 after 22 days.

This experiment affords a preliminary proof that the blue tick is not a carrier of piroplasma mutans, although it transmits redwater (piroplasma bigeminum).

The injection of blood containing piroplasma mutans proved that all six animals were susceptible to piroplasma mutans, since the injection of blood caused the appearance of the parasite.

Experiment No. 3.

Susceptible heifers injected (1) *with piroplasma bigeminum exclusively*, (2) *infested with larval blue ticks feeding on animals containing piroplasma bigeminum and piroplasma mutans in their blood, and* (3) *injected with blood containing piroplasma bigeminum and piroplasma mutans.*

These experiments were intended in the first instance to note whether the blue tick would act as a carrier of piroplasma mutans; if so, it would then infect animals susceptible to piroplasma mutans.

If these animals failed to show a reaction consequent on the infestation, yet subsequent injections introduced the two piroplasms, it would prove that the blue tick is not a carrier of the disease.

"*A*," *Ox* 358.—About one year old; directly imported from Cape Colony and susceptible to redwater; was injected on the 6th July, 1906, with 10 c.c. blood of calf 359, containing piroplasma bigeminum exclusively in its blood. A temperature reaction ensued, and on the 20th day poikilocytosis and piroplasma bigeminum were noticed. The reaction subsided about the 25th day, and a normal curve resulted. On the 49th day after this inoculation—24th August—the ox was infested with numerous blue tick larvæ of ox 347, whose blood in previous experiments had proved to contain both piroplasma bigeminum and piroplasma mutans. Nothing resulted from the tick infestation; accordingly on the 19th October—56 days after the tick infestation—ox 358 was injected with 5 c.c. blood of heifer 316—immune to piroplasma bigeminum and piroplasma mutans; therefore containing both these piroplasms in its blood.

Nothing resulted from this inoculation until the 28th day, when one ring was noticed. These rings were very rarely met with up to the 38th day, on which date a slight febrile reaction occurred and the piroplasms slightly increased in numbers. The experiment was discontinued on the 18th December, 1906—60 days after the injection of blood of heifer 316.

"*B*," *Calf* 387.—This calf belonged to the same lot which subsequently proved to be immune against redwater.

Injected on the 6th July with 10 c.c. blood of calf 359 immune to redwater. Nothing resulted; the temperature remained normal, and examinations of blood constantly proved negative. Fifty-four days after this inoculation—29th August—387 was infested with numerous blue tick larvæ of heifers 314 and 316—whose blood contained both piroplasma mutans and piroplasma bigeminum. Nothing occurred from this infestation; the temperature again remained normal, and microscopical examinations constantly proved negative.

On the 4th November—67 days after the tick infestation—the animal was injected with 5 c.c. blood of heifer 316, immune to piroplasma bigeminum and piroplasma mutans. Negative results from this inoculation until the 7th December—33rd day—when rings and rods were noticed, but were very rare during the following days. The experiment was discontinued on the 18th December—44 days after the injection of blood of 316.

"*C*," *Heifer* 394.—Imported from Aliwal North, and therefore susceptible to redwater. Injected on the 24th August, 1906, with 10 c.c. blood of calf 359, whose blood contained piroplasma bigeminum exclusively. The temperature reaction commenced on the 10th day, and on the following day piroplasma bigeminum was noticed. This piroplasm was present for five days, when a slight poikilocytosis was observed, which continued up to the 20th day. A second reaction was noticeable between the 33rd and 45th days, although nothing particular was noticed in the blood. On the 54th day after the first inoculation—*i.e.*, 17th October, 1906—394 was infested with numerous blue tick larvæ of ox 347—an animal which contained piroplasma bigeminum and piroplasma

mutans in its blood. On the 13th day after infestation a temperature reaction was noticed which passed over after three days, but was succeeded by another curve, at the beginning of which piroplasma bigeminum and spirillum were noticed (ox 347 also being infected with spirillum). From the 30th to 37th day piroplasma bigeminum was noticed daily, and a very slight febrile reaction ensued. The temperature curve now remained normal.

Sixty-three days after the tick infestation—19th December—394 was injected with 10 c.c. blood of heifer 316, which contained both piroplasma bigeminum and piroplasma mutans in its blood. Twenty-four days after the injection the first rod-shaped parasite was seen. The parasites were now daily noticed in slightly increasing numbers, and accompanied with the appearance of a slight poikilocytosis and a considerable rise of temperature.

"*D*," *Heifer* 395.—Imported from Aliwal North, and therefore susceptible to redwater. Injected on the 24th August, 1906, with 10 c.c. blood of calf 359 immune to ordinary redwater. Temperature reaction began on the 8th day; piroplasma bigeminum was noticed on the 11th day only. The temperature curve continued until the 15th day, on which date marginal points and the lesions of poikilocytosis made an appearance. Poikilocytosis was noticed up to the 26th day, and between the 31st and the 38th days another temperature disturbance was noticeable, but examinations of blood proved negative. The temperature now remained normal until the 17th October, 1906, this being the 54th day after inoculation.

The animal was now infested with numerous blue tick larvæ of ox 347. On the 20th day after this infestation a temperature reaction commenced, on which day and the day following spirillum was noted—347 being also infected with spirillum. On the 31st day, and coinciding with a slight rise of temperature, piroplasma bigeminum was noticed; it was also present on the 31st, 35th and 44th days. Subsequent to this the lesions of a slight poikilocytosis were occasionally seen. Sixty-three days after the tick infestation the animal was injected with 10 c.c. blood of heifer 316—an animal which contained both piroplasma bigeminum and piroplasma mutans in its blood. Twenty-six days later a slight poikilocytosis was noticed, and on the 33rd day the first rod-shaped parasite was seen. These rod-shaped parasites were now daily noticed, accompanied by a slight poikilocytosis up to the 31st day.

"*E*," *Heifer* 396.—Imported from Aliwal North, and therefore susceptible to redwater.

Injected on the 24th August, 1906, with 10 c.c. blood of heifer 358, an animal infected with piroplasma bigeminum exclusively. A rise of temperature ensued on the 7th day; on the 9th day piroplasma bigeminum appeared, and was noticed daily for the three following days. On the 4th day poikilocytosis was well pronounced; polychromatic cells and basophile granulations were also noted. These blood changes were noticed for a considerable time, and were present at the time of the second reaction, which occurred between the 30th and the 36th day. No piroplasms were present during this period. The temperature again became normal. On the 54th day after inoculation—17th

October—the animal was infested with numerous blue tick larvæ of ox 347—an animal infected with both piroplasma bigeminum and piroplasma mutans. On the 19th day a slight rise of temperature became noticeable, and three days later spirillum was noticed, succeeded by the lesions of poikilocytosis. This slight poikilocytosis was constantly noticed up to the 63rd day slightly decreasing, whilst the temperature remained normal. On this day—19th December—the animal was injected with 10 c.c. blood of heifer 316, containing piroplasma bigeminum and piroplasma mutans. The temperature remained normal, and on the 21st day the first rod-shaped parasite was seen. Again, on the 24th day, another one was noticed, and from the 26th day onwards they slightly increased, together with a slight rise of temperature.

On the 23rd January, 1907, the animal died accidentally, it having become hoven.

Conclusions.

All these five animals—Nos. 358, 387, 394, 395 and 396—were treated in the same way, and the experiment had a similar result.

They were injected in the first instance with blood of an animal which was infected exclusively with redwater (piroplasma bigeminum).

From 49 to 54 days after this injection they were infested with numerous blue tick larvæ previously feeding on animals suffering from both piroplasma bigeminum and piroplasma mutans. In two instances—"*C*" and "*D*"—piroplasma bigeminum made its appearance, probably due to this infestation, and in the three cases—"*C*," "*D*," and "*E*"—was accompanied by the infection of spirillum (the animal from which the ticks were taken also being infected with spirillum).

If the blue tick is the carrier of piroplasma mutans it would have communicated the infection to these five animals. It did not do so within 56 to 67 days, when the animals were injected with blood containing both piroplasma bigeminum and piroplasma mutans, and, as a result of this injection, piroplasma mutans appeared with the typical incubation time (see appendix).

APPENDIX "A."

Animals.	Numbers of Days which elapsed between			
	Infection of piroplasma bigeminum exclusively and infestation of blue tick larvae.	Infestation of blue tick larvae and infection of both piroplasma bigeminum and piroplasma mutans.	Infection of both piroplasms and the appearance of rods and rings—piroplasma mutans.	Injection of blood containing piroplasma bigeminum and injection of blood containing both piroplasma bigeminum and piroplasma mutans.
358	49	56	28	105
387	54	67	33	121
394	54	63	24	117
395	54	63	33	117
396	54	63	21	117

Experiment No. 4.

To prove that the injection of blood of an animal immune to redwater which contracted the disease from ticks, is not followed by the appearance of piroplasma mutans.

About two years ago I forwarded blue ticks—taken from an animal which was suffering from ordinary redwater—to Professor Sir J. M'Fadyean, of London. These ticks were then placed on an animal "X," with the result that it passed through a typical reaction and shewed piroplasma bigeminum in its blood. The animal recovered, and its blood was utilised for the injection into heifer 429 in England, with the result that this animal also passed through a typical ordinary redwater reaction, and piroplasma bigeminum appeared. Heifer 429 was then sent to South Africa (see also article "B," Experiments with English and South African Redwater), and soon after its arrival at this laboratory the injection into 426 was made.

"A," *Heifer* 426.—Two years old; imported from Aliwal North and therefore susceptible to ordinary redwater. Injected on the 22nd December, 1906, subcutaneously with 10 c.c. blood of English heifer No. 429.

Slight rise on the 10th day, reaching 103.4 twenty-four hours later, when the presence of piroplasma bigeminum was noted, and remained for the following three days. The temperature shewed considerable fluctuations and marked differences between the morning and evening records were noted until the 77th day after this injection. On this date—8th March, 1907—heifer 426 was injected with 10 c.c. blood of heifer 409, immune to piroplasma mutans. No distinct reaction noticed, but the evening record remained fairly high, and poikilocytosis was occasionally present. Piroplasma mutans present for the first time on the 26th day after injection, and again noted four and seven days later.

All further examination negative, and the experiment was discontinued from the 59th day—136 days after injection of blood of English heifer No. 429.

Injections with blood of heifer 426, *an animal immune to piroplasma bigeminum.*

"B," *Heifer* 421.—About two years old; imported from Aliwal North, and therefore susceptible to redwater. Injected on the 30th January, 1907, subcutaneously with 10 c.c. blood of heifer 426 (compare Experiment 4, "A").

Sharp rise noted from the sixth day, reaching 105.6 two days later, followed by the appearance of piroplasma bigeminum for the first time on the tenth day. The lesions of a strong poikilocytosis noted five days later and from this day until the 36th an irregular temperature record ensued. On this latter date polychromatic cells, accompanied with the lesions of a strong poikilocytosis, were noted. Next morning, 8th March, 1907, heifer 421 was injected subcutaneously with 10 c.c. blood of heifer 409—an animal containing both piroplasms in its blood (see Experiment 2, "F"). Reaction from the second day, accompanied with poikilocytosis, and reaching 107 on the 8th

and 9th days, when piroplasma bigeminum was noted on both occasions. The temperature now slightly fell, still accompanied with poikilocytosis, and on the 25th day piroplasma mutans was present for the first time, together with poikilocytosis and marginal points. These lesions were again noted three days later, and on the 30th, 31st and 34th days, Basic cells also being present on the 31st day.

"*C*," *Heifer* 423.—About two years old; directly imported from Aliwal North, and therefore susceptible to ordinary redwater. Injected on the 30th January, 1907, subcutaneously with 10 c.c. blood of heifer 426 (compare Experiment 4, "*A*"). Reaction from the 6th day, reaching 103.8 three days later when piroplasma bigeminum was noted. The temperature remained fairly high until the 36th day. On the 37th day—8th March, 1907—heifer 423 was injected with 10 c.c. blood of heifer 409 (compare Experiment 2, "*F*"), immune to piroplasma mutans and piroplasma bigeminum. After an incubation time of 25 days, piroplasma mutans, accompanied with slight poikilocytosis, appeared, and were again noted on 27th, 31st and 34th days, during which time the reaction was very marked. Piroplasma mutans alone was present in fair numbers on the 32nd day. The experiment was discontinued on the 7th May, 1907, 60 days after the injection of 409.

"*D*," *Heifer* 424.—About two years old; imported from Aliwal North, and therefore susceptible to redwater. Injected on the 30th January, 1907, subcutaneously with 10 c.c. blood of heifer 426 (see Experiment 4, "*A*"). Slight reaction from the 6th day, reaching 104 on the 10th and 11th days, and followed by the lesions of poikilocytosis on the 12th and 15th days. On the 30th day the temperature recorded 105.4, and this was again followed by poikilocytosis on the next day and accompanied with polychromatic cells on the 35th day. Two days later heifer 424 was injected subcutaneously with 10 c.c. blood of heifer 409 (compare Experiment 2, "*F*") and immune to piroplasma bigeminum and piroplasma mutans. Slight poikilocytosis and piroplasma mutans noted for the first time on the 25th day, and two days later these lesions were again present, accompanied with marginal points. Slight poikilocytosis and piroplasma mutans again noted on the 31st and 34th days. The temperature consistently remained between 99 and 104 until the 101st day, when the experiment was discontinued.

Conclusions.

When blue ticks obtained from a South African beast suffering from ordinary redwater were forwarded to England and placed on an animal, they transmitted piroplasma bigeminum; all the animals which were subsequently inoculated in South Africa with blood from a following generation of this strain developed piroplasma bigeminum exclusively, thereby proving that piroplasma mutans was not carried to England by the blue tick. The three South African animals injected with piroplasma mutans developed this infection within the typical period, thus proving that piroplasma mutans and piroplasma bigeminum are not connected with each other in any way.

Experiment No. 5.

SPONTANEOUS CASES OF PIROPLASMA MUTANS.

A.—Cases originating in Cape Colony.

"A," *Calf* 380.—This is an animal belonging to the Capetown lot, which was considered susceptible to piroplasma bigeminum, but experiments proved the presence of this parasite in its blood, therefore indicating that the animal was immune against redwater.

Injected subcutaneously on the 6th June, 1906, with 5 c.c. blood of calf 378, being one of the same batch of animals. Negative results, the temperature remained normal, and microscopical examinations for 83 days—until August 28th—proved negative. At the beginning of September, 1906, rings (piroplasma mutans) were noticed in scanty numbers, and disappeared after about ten days. The examination of smears was continued, but again without any positive results.

This calf was used for calf vaccine lymph on the 19th October, 1906—135 days after the first inoculation. The reaction from the vaccination concluded on the 4th November, and on that date 380 was inoculated with blood of heifer 316—containing piroplasma bigeminum and piroplasma mutans. Eight days after this inoculation rings and rods were again noticed. They became rarer and rarer during the following days, but were still present when the experiment was discontinued on the 18th December, 1906.

In this particular case I do not consider that the reappearance of piroplasma mutans is due to the inoculation of blood of 316, but to the heavy reaction consequent on the vaccination.

"B," *Heifer* 411.—Injected on the 16th October, 1906, with 5 c.c. blood of the former animal No. 380, which, as shewn, contained piroplasma mutans. The object of the experiment was to note whether we had perhaps to deal with a pure infection of piroplasma mutans.

Following on the injection, on the 11th day a reaction ensued, and for the next three days piroplasma bigeminum was encountered. The usual changes of a piroplasma infection were noticed in the form of poikilocytosis.

On the 45th day after inoculation, rings and rods were noticed. They were, however, very rare, and hardly made an impression on the course of the temperature, which only shewed a slight rise in the morning.

The experiment was discontinued on the 60th day—16th of January, 1907.

Experiment No. 6.

B.—Cases originating in the Transvaal.

On the 1st October, 1906, Government Veterinary Surgeon Lindsay, of Middelburg, forwarded some smears of a dead cow, which although grazing in an East Coast fever infected area was not supposed to have died of this disease, but as the result of an accident.

Microscopical examinations proved the presence of endoglobular parasites corresponding to the description of piroplasma mutans, but since the animal was in an infected area a reservation was made as to the cause of death in order to make further investigations of the remaining in contact animals. Smears from two healthy animals running together with the one which had died were made, and examinations also shewed endoglobular parasites. It was now decided to prove whether this was a case of a pure infection of piroplasma mutans, and accordingly the animals were tapped by the Government Veterinary Surgeon, the blood forwarded to the laboratory on the 3rd November, and the following injections into calves 416 and 417 were made on the next day:—

"*A*," *Calf* 416.—Born on the station, and injected as above with 5 c.c. blood from a red ox. No rise of temperature or the presence of any parasites noticed for the first 28 days. On the next day marginal points appeared, and on the 30th day rings and rods (piroplasma mutans) were noticed for the first time. The symptoms of poikilocytosis increased; marginal points, rings and rods also increased, whereas the temperature showed fluctuations, but during the increase of piroplasma mutans a distinct rise ensued. The parasites were present up to the 61st day after inoculation in rare numbers, and on that day—January 4th, 1907—the examination was discontinued.

"*B*," *Calf* 417.—This calf was injected as above. Nothing happened until the 18th December, when one piroplasma bigeminum was noticed. On the 27th day rings and rods were seen. They were, however, very rare, and hardly increased up to the 25th December, when the examinations were discontinued.

In December, 1906, Mr. Dunning, then Government Veterinary Surgeon at Zeerust, forwarded blood preparations from a sick calf, and microscopical examinations revealed the presence of piroplasma mutans exclusively. It was then thought that this might prove to be a case of pure piroplasma mutans infection, and accordingly the calf was tapped, blood forwarded to this laboratory, and the following injection was made on the 21st December, 1906.

"*C*," *Heifer* 425.—About two years old; imported from Aliwal North. Injected as above with 10 c.c. blood from Zeerust calf. Irregular reaction from date of injection. Poikilocytosis noted on the 26th and 28th days, and on the 31st day, accompanied with flagellated forms for the first time. The rod and ring forms were also noted the following day. Slight poikilocytosis, ring and flagellated forms were occasionally seen until the 50th day, the temperature on the previous day recording 105. Experiment discontinued on the 181st day after inoculation.

Experiment No. 7.

To shew that blood containing piroplasma bigeminum and piroplasma mutans when injected into cattle susceptible to ordinary redwater does not always cause the former parasite to appear.

As heifer 425 did not shew piroplasma bigeminum consequent on the injection of blood from the Zeerust calf, the supposition that this might prove a pure case of piroplasma mutans infection received support. Accordingly the following experiments were made:—

Injections with blood of heifer 425, supposed to be a case of pure piroplasma mutans.

"*A*," *Heifer* 434.—About two years old, and imported from England. Injected on the 31st January, 1907, subcutaneously with 10 c.c. blood of heifer 425. A sharp rise noted on the 7th day, reaching 105.4 twenty-four hours later, and accompanied with poikilocytosis. Spirillum noted on the 14th day, and from the 23rd day a typical reaction was noted, rings being noted for the first time on this date. Piroplasma mutans, marginal points and poikilocytosis were noted on the 25th, 27th, 28th and 29th days. Basic and nucleated cells together with marginal points again accompanied with polychromatic cells. The heifer died on the 34th day, 5th March, 1907.

Post-mortem Report.

Condition:—Good. Rigor mortis not completely set in, blood still running from axillary vein. Beef somewhat pale. Subcutaneous and intra-muscular tissue yellow.

Lungs:—Interstitial emphysema. Lungs pale. Slight oedema.

Heart:—Pericard contained a little liquid. A few petechiae in heart bag. Epicard almost brick red. Endocard normal.

Spleen:—Enlarged to about 1½ times normal size. Pulpa soft.

Kidneys:—Normal but somewhat pale. Urine pale.

Bladder:—Gall bladder slightly distended; contained thick green bile.

Stomach:—Third stomach soft; mucosa pale, with bile stained streaks.

Intestines:—A few superficial haemorrhagic small spots on caecum. Mucosa of small intestines covered with a layer of a slight yellow tinge.

"*B*," *Heifer* 422.—About two years old; imported from Aliwal North. Injected on the 30th January, 1907, subcutaneously with 10 c.c. blood of heifer 425. Slight reaction from the 15th day. All examinations negative until the 24th day, when poikilocytosis was noted, and accompanied the following day with piroplasma bigeminum. Piroplasma mutans noted for the first time on the 32nd day, and again present two days later, together with poikilocytosis. Discontinued on the 71st day.

"*C*," *Heifer* 401.—About two years old; imported from Aliwal North. Injected on the 30th January, 1907, subcutaneously with 10 c.c. blood of heifer 425. Slight reaction from the 6th day and a secondary one from the 19th day. All examinations negative until the 24th day, when the rings and flagellated forms accompanied with poikilocytosis appeared for the first time. These lesions were frequently present during the first four days, when a sharp rise to 107.4 on the 33rd day was accompanied with piroplasma bigeminum, which was also noted on the two following days. The temperature regained normal on the 42nd day, polychromatic cells being noted four days previously. The experiment was discontinued on the 69th day.

"*D*," *Heifer* 420.—About two years old; imported from Aliwal North. Injected on the 31st January, 1907, subcutaneously with 10 c.c. blood of heifer 425. Reaction from the 17th day, poikilocytosis being present, and five days later flagellated and ring forms were noted for the first time, and again on the following day, accompanied with piroplasma bigeminum.

Piroplasma bigeminum, rings, basic and polychromatic cells were noticed on the 24th day, and the latter lesions were again present on the 27th, 29th and 34th days. Another reaction noted from the 53rd day, accompanied with poikilocytosis and piroplasma mutans six days later. The experiment was discontinued on the 77th day.

"*E*," *Heifer* 445.—About two years old; imported from Aliwal North. Injected subcutaneously on the 26th March, 1907, with 10 c.c. blood of heifer 425. Poikilocytosis occasionally noted from the 22nd to the 38th day, and on the following day accompanied with piroplasma mutans; these lesions again present on the 43rd and 45th days, and from this date a typical reaction was noted, lasting for 15 days, and during which time piroplasma mutans was present on the 54th, 56th, 57th and 58 days. The experiment was discontinued on the 85th day.

"*F*," *Heifer* 418.—Injected on the 26th March, 1907, subcutaneously with 10 c.c. blood of heifer 425. Irregular reaction. Poikilocytosis noted on the 26th day, and piroplasma mutans on the 33rd day. Poikilocytosis and piroplasma mutans again present on the 35th, 37th, 40th, 43rd and 47th days.

"*G*," *Heifer* 419.—About two years old, and imported from Aliwal North. Injected on the 26th March, 1907, subcutaneously with 10 c.c. blood of heifer 425. Rise of temperature noted six days later, and on the 9th day piroplasma bigeminum was noted. Piroplasma mutans present on the 24th day, together with poikilocytosis, and were both frequently noted until the 47th day.

"*H*," *Heifer* 449.—About two years old; imported from Aliwal North. Injected on the 26th March, 1907, subcutaneously with 10 c.c. blood of heifer 425. Irregular temperature, poikilocytosis being noted on the 22nd, 23rd and 27th days, accompanied with piroplasma mutans for the first time on the 30th day. Piroplasma mutans, together with poikilocytosis, were noticed on the 33rd, 36th, 37th, 39th, 42nd and 44th days. Experiment discontinued on the 85th day.

"*I*," *Heifer* 400.—About two years old, and imported from Aliwal North. Injected on the 30th January, 1907, with 10 c.c. of heifer 425.

Reaction from the 13th day, and 10 days later flagellated forms noted. The lesions of a piroplasma mutans infection, frequently present from the 24th day, this piroplasm being noticed on the 30th and 35th days.

As no piroplasma bigeminum appeared in this animal, and in order to prove conclusively that the Zeerust calf was not a pure case of piroplasma mutans infection, heifers 448 and 453 were injected.

Injections with blood of heifer 400.

"*J*," *Heifer* 448.—About two years old, and injected on the 26th March, 1907, subcutaneously with 10 c.c. blood of 400.

Sharp rise from the 6th day, reached 106 four days later, and on the 15th day basic and polychromatic cells were present, when piroplasma bigeminum was noted. This piroplasm was again noted two days later. Piroplasma bigeminum again noted on the 24th day. Poikilocytosis was occasionally noticed until the 40th day, and two days later piroplasma mutans appeared, together with the lesions of poikilocytosis. Piroplasma mutans and poikilocytosis were again present on the 45th day, and the experiment was discontinued from the 81st day.

"*K*," *Heifer* 453.—About two years old, and imported from Aliwal North. Injected on the 26th March, 1907, subcutaneously with 10 c.c. blood of heifer 400. A sharp rise to 106 on the 7th day was followed by the appearance of piroplasma bigeminum for the first time. The temperature record now shewed sharp oscillations for the next ten days, accompanied with basic, nucleated and polychromatic cells. Piroplasma bigeminum, basic and nucleated cells were again noted on the 22nd day. Basic and polychromatic cells, together with poikilocytosis, were occasionally noted for the next 16 days, and on the 39th day piroplasma mutans appeared for the first time. From the 48th day a febrile reaction was noted, the temperature reaching 106 on the 55th day, and accompanied with piroplasma mutans. The same record was observed on the 59th day, and again piroplasma mutans was present. Experiment was discontinued on the 80th day.

Conclusions from animals 425, 434, 422, 401, 420, 445, 418, 419, 449, 400, 448 *and* 453.

The inoculation with blood in which microscopically only piroplasma mutans was noted produced a reaction in animals Nos. 425 and 434 without the appearance of piroplasma bigeminum, but a subsequent inoculation into animals 422, 401, 420, 419, 448 and 453 proved the presence of piroplasma bigeminum, which, as these animals were susceptible to ordinary redwater, can only be traced to this injection of blood. Therefore, although the injection of blood containing piroplasma mutans is not always followed by the appearance of piroplasma bigeminum, no conclusion can be drawn as to the presence of piroplasma bigeminum in the injected animal, unless the experiment is carried out on a sufficient number of animals.

In the foregoing experiments it has been shewn that an animal can be exclusively infected with piroplasma bigeminum either by a subcutaneous injection of blood obtained from an animal immune against redwater or by blue ticks originating from animals infected with both piroplasma bigeminum and piroplasma mutans, but in no instance was piroplasma mutans transmitted by the blue tick. In every case where piroplasma bigeminum appeared, a subsequent injection with blood containing piroplasma bigeminum and piroplasma mutans caused piroplasma mutans to appear within the typical incubation time. This fact has to be regarded as a conclusive proof of the duality of the two piroplasms.

Proof has also been given that notwithstanding the non-appearance of piroplasma bigeminum after animals were injected with blood containing piroplasma mutans, yet piroplasma bigeminum proved to be present when this blood was subsequently tested.

"B."—EXPERIMENTS WITH ENGLISH AND SOUTH AFRICAN REDWATER.

In order to overcome the mortality caused by ordinary redwater when exposing imported cattle on the South African veld, I decided to conduct some experiments in this connection, and was fortunate in obtaining the assistance of Mr. Stockman, Principal Veterinary Surgeon of England.

The experiments were performed with a three-fold object, namely, to test (1) whether the English and South African redwater are identical, (2) if English cattle immunised against English redwater would thereby acquire any immunity against the ordinary redwater of South Africa, and (3) if English heifers inoculated in England with South African redwater would be immune against our disease when exposed to natural infection in South Africa.

Accordingly six heifers were purchased on our behalf by Mr. Stockman, and were treated by him in England. One lot were injected with English redwater, the second batch with South African redwater, and the remaining two heifers with both English and South African redwater.

I am greatly indebted to Mr. Stockman for making careful examinations of these animals, and it is from his notes that the following particulars of temperatures and blood examinations have been obtained:—

Experiment No. 1.

English heifers injected with English redwater.

"A," *Heifer* 428.—Injected in England subcutaneously on the 25th July, 1906, with 5 c.c. defibrinated blood of a heifer (which had previously been inoculated with blood obtained from a natural case of redwater in Hampshire, but which did not react in any marked degree, and it is highly probable that she did not contract the infection).

Heifer 428 did not shew any reaction, and all smears examined from the date of injection to the 1st September gave negative results.

On the 1st September, 1906, 428 received an injection of 10 c.c. defibrinated blood from an English cow which had recovered from a natural attack of English redwater. Temperature rose on the 8th day to 105 F. in the morning and 106 in the evening, returning next day to normal. On the 24th day it reached 104.6 in the evening, but fell again to normal two days later.

Examinations of blood preparations from the 8th to 26th September gave negative results, and the blood count on the 4th October, 1906, shewed the number of red cells to be between six and seven millions per c.mm.

"*B*," *Heifer* 430.—Injected in England with 5 c.c. defibrinated blood of an English heifer (which had previously been inoculated with English redwater, but the injection probably failed to infect the beast).

430 did not shew any reaction, and no piroplasms were found in the blood preparations.

On the 1st September, 430 was inoculated subcutaneously with 10 c.c. defibrinated blood of an English cow which had recovered from a natural attack of English redwater.

Temperature of 430 rose to 102 on the 6th day and to 104 in the evening of the 8th day. Smears examined on the morning of this latter date shewed distinct, but only small numbers of piroplasms, and the evening examination shewed a few ring forms; the temperature now fluctuated between 102.6 and 105.2 for the next two days, and regained normal on the 11th September. Another, but slight, reaction ensued from the 23rd to the 28th September.

Experiment No. 2.

English heifers injected with South African redwater.

"*A*," *Heifer* 429.—Injected in England on the 1st September, 1906, with 5 c.c. defibrinated blood of an English heifer (which had been infested with the infected blue ticks I sent to Professor Sir J. M'Fadyean in 1905, and as a result developed South African redwater and recovered).

On the 9th day the temperature of 429 rose to 103, but examination of smears were negative. On the 10th day temperature recorded 105.2 in the evening, and piroplasms were found in the smears. The temperature fluctuated for the next four days, but returned to normal on the 15th September. Piroplasms were again noted on the 11th day.

"*B*," *Heifer* 432.—Injected on the 1st September, 1906, with 5 c.c. defibrinated blood from the English beast which had been infected with blue ticks and contracted South African redwater.

Temperature of 432 rose to 103 on the 6th day, but examination of smears were negative; regained normal on the 7th to 9th days, but recorded 105 on the 10th day, when blood examinations were again negative. Piroplasms were noted for the first time, and only on the following day. Temperature remained high for the next three days, and regained normal on the 16th September.

Experiment No. 3.

English heifers inoculated with English and South African redwater.

"*A*," *Heifer No.* 431.—Injected on the 25th July in England with 5 c.c. blood from an English heifer (which had previously been inoculated with English redwater, but this injection apparently did not cause the beast to contract the disease).

The temperature of 431 remained normal, and all blood examinations gave negative results.

On the 1st September 431 was injected subcutaneously with 10 c.c. blood of an English cow which had recovered from a natural attack of English redwater.

Temperature rose to 104.2 in the morning of the 8th day, and to 106 in the evening. Blood examinations on this date were negative. Temperature remained high for the next two days, but returned to normal on the 10th September.

On the 24th September 431 was injected subcutaneously with 10 c.c. defibrinated blood of an English heifer (which had been infested with infected South African blue ticks, causing her to contract South African redwater).

431 shewed a rise of temperature, consequent on this inoculation, to 105 in the evening of the 1st October, 1906—the 7th day. Piroplasms were noted in the blood on this date, and a high temperature was recorded for the next three days.

"*B*," *Heifer* 433.—Injected in England on the 5th July subcutaneously with 5 c.c. defibrinated blood of an English beast (which had been inoculated with English redwater, but apparently did not contract the infection).

The temperature of 433 remained normal, and all blood examinations were negative.

On the 1st September, 1906, 433 was injected with 5 c.c. defibrinated blood of the English beast, which had contracted South African redwater from the infestation of infected blue ticks. Temperature of 433 rose to 103 on the 6th day, but examinations of blood did not reveal any piroplasms. The temperature now fluctuated for the next four days, and no piroplasms were seen until the 11th and 12th days, when the temperature regained normal.

A short secondary reaction noted from the 22nd day, lasting for a few days, but no piroplasms were found.

On the 24th September, 433 was injected with 10 c.c. defibrinated blood of a beast which had recovered from a natural attack of English redwater.

Ten days later 433 shewed a slight reaction, and on this date and the following days piroplasms were present. Temperature regained normal on the 6th October.

Notes on heifers 428 *and* 430.

From Mr. Stockman's notes on the behaviour of heifer 428 in England, I am not inclined to consider it as immune against English redwater in view of the atypical reaction and the absence of piroplasms.

Heifer 430 undoubtedly underwent an English redwater reaction, and should therefore be immune against this disease.

Notes on heifers 429 *and* 432.

The injection of English heifers with a strain of virus originating from infected blue ticks sent from the Transvaal and passing through an English beast caused, in both instances, the appearance of piroplasms accompanied with a typical South African redwater reaction. Therefore these two heifers should be immune against South African redwater.

Notes on heifers 431 *and* 433.

The injection of English redwater into heifer 431 did not cause the appearance of piroplasms, and the reaction was atypical. A subsequent injection of South African redwater caused the appearance of piroplasms in the English heifer, together with a temperature reaction.

Heifer 433 was injected with South African redwater in the first instance, and as a result shewed piroplasms, accompanied with a temperature reaction.

Twenty-four days later it was injected with English redwater, and a slight reaction ensued, accompanied with piroplasms.

Heifer 433 should, therefore, have acquired immunity against both English and South African redwater.

Conclusions.

It would be seen from these notes that of four animals inoculated with English redwater, two failed to react or to shew piroplasms, and in the other two instances a reaction ensued, accompanied with the appearance of piroplasms. Therefore it is safe to say that English redwater is not always inoculable, and differs in this respect from South African redwater.

EXPERIMENT No. 4.

To note whether (1) *South African animals, susceptible to ordinary redwater, contract English redwater when injected with blood from animals previously inoculated with this disease, and* (2) *whether these South African animals prove immune against ordinary redwater when injected with piroplasma bigeminum.*

(The South African animals used in this experiment were born and bred in Cape Colony, in a district free of ordinary redwater, directly imported to the Transvaal; hence they were susceptible to the disease.)

Heifers Nos. 400, 418, 421 and 422 were all injected on the 13th December, 1906, with blood of English heifer 428. Heifer 428 (compare Experiment 1 A) had shewn a temperature reaction consequent on the inoculation with English redwater, but no piroplasms were noted in the blood.

These South African heifers were subsequently tested on their immunity against ordinary redwater by the injection of a strain of virus emanating from a natural case of ordinary redwater, complicated with piroplasma mutans. (See also "Further Notes on Piroplasma Mutans," Experiment No. 6 C, Heifer 425.)

"A," *Heifer* 400.—A two-year-old from Aliwal North, and susceptible to ordinary redwater. Injected on the 13th December, 1906, subcutaneously with 10 c.c. blood of heifer 428. As there were no results from this injection, heifer 400 was again injected subcutaneously on the 3rd January, 1907, with 50 c.c. blood of heifer 428. Temperature remained quite normal, and, with the exception of the presence of poikilocytosis on rare occasions, all blood examinations were negative.

Tested on immunity against ordinary redwater by the subcutaneous injection of 10 c.c. blood of heifer 425, containing piroplasma bigeminum, on the 30th January, 1907. Reaction from the 5th day, reaching 104.6 four days later, and remaining high for the next three weeks. Poikilocytosis and the lesions of piroplasma mutans appeared, but piroplasma bigeminum was not seen.

On the 26th April, 1907, 400 was injected with 10 c.c. blood of heifer 435, which was an imported English heifer, and had been

rendered immune against ordinary redwater. This injection did not cause any temperature reaction in 400, nor did piroplasma bigeminum appear.

"*B*," *Heifer* 418.—Two-year-old heifer from Aliwal North, and susceptible to South African redwater.

Injected on the 13th December, 1906, subcutaneously with 10 c.c. blood of heifer 428. No reaction; all blood examinations proved negative. Accordingly on the 3rd January, 1907, heifer 418 was again injected subcutaneously with 50 c.c. blood of heifer 428. The temperature remained quite normal, and no piroplasms were found in the blood smears. Spirillum appeared on the 3rd day after the second injection, and five days later the lesions of poikilocytosis were noted, remaining for another two days.

Tested on the 26th March, 1907, by a subcutaneous injection of 10 c.c. blood of heifer 425, an animal which contained piroplasma bigeminum and piroplasma mutans in its blood. A slight temperature reaction followed, and on the 26th day piroplasma bigeminum appeared.

"*C*," *Heifer* 421.—Two-year-old heifer from Aliwal North, and susceptible to ordinary redwater.

Injected on the 31st December, 1906, intrajugularly with 5 c.c. blood of heifer 428. As no reaction ensued, and all blood examinations were negative, heifer 421 was again injected subcutaneously on the 3rd January, 1907, with 50 c.c. of heifer 428. With the exception of a sharp rise to 104 on the 3rd day after this latter injection the temperature remained normal, and no piroplasms were noted in the blood.

Tested on the 30th January, 1907, by subcutaneous injection of 10 c.c. blood of heifer 426, containing piroplasma bigeminum.

Reaction from the 5th day, piroplasma bigeminum being noted four days later.

"*D*," *Heifer* 422.—Injected on the 13th December, 1906, subcutaneously with 10 c.c. blood of heifer 428.

Temperature remained normal until the 17th day, when a sharp rise occurred, reaching 105.2, but all microscopical examinations were negative. Heifer 422 was again accordingly injected on the 31st January, 1907, subcutaneously with 50 c.c. blood of heifer 428. Again no reaction ensued, the temperature consistently remaining about 102 to 103. All examinations negative, with the exception of the presence of rare Trypanosoma theileri on the 6th January, 1907. Tested on the 30th January, 1907, by subcutaneous injection of 10 c.c. blood of heifer 425, containing piroplasma bigeminum and piroplasma mutans.

Reaction from the 15th day, reaching 104.2 the following day, and remaining high for about two weeks. Piroplasma bigeminum noted on the 25th day.

The following South African cattle Nos. 401, 419, 420, 423 and 424 were all injected with blood of English heifer No. 430. This heifer (compare Experiment 1, "*B*") had been inoculated in England with English redwater, and as a result gave a temperature reaction, accompanied with the presence of piroplasms.

"*E*," *Heifer* 401.—A two-year-old heifer, from Aliwal North, and susceptible to ordinary redwater.

Injected on the 13th December, 1906, subcutaneously with 10 c.c. blood of English heifer 430. As this inoculation failed to cause a reaction, and no piroplasms were noted in the blood, heifer 401 was reinjected on the 3rd January, 1907, subcutaneously with 50 c.c. blood of 430. Again no temperature reaction ensued, and all blood examinations were negative.

Tested on the 30th January, 1907, by subcutaneous injection of 10 c.c. blood of heifer 425, containing piroplasma bigeminum.

Reaction from the 6th day, returning to normal seven days later. Secondary reaction from the 19th day, reaching 107.4 on the 33rd day, and during which piroplasma bigeminum appeared.

"*F*," *Heifer* 419.—A two-year-old heifer from Aliwal North, and susceptible to ordinary redwater.

Injected on the 13th December, 1906, subcutaneously with 10 c.c. blood of heifer 430.

No temperature reaction, and all examinations of blood negative. Heifer 419 was then reinjected on the 3rd January, 1907, subcutaneously with 50 c.c. blood of heifer 430, with the result that the temperature remained normal, and no piroplasms or blood changes were noted.

Tested on 26th March, 1907, by subcutaneous injection of 10 c.c. blood of heifer 425, an animal immune against ordinary redwater. Reaction six days later, reaching 104.2 on the 3rd April, 1907. Piroplasma bigeminum noted on the 9th day.

"*G*," *Heifer* 420.—A two-year-old heifer from Aliwal North, and susceptible to ordinary redwater.

Injected on the 13th December, 1906, subcutaneously with 10 c.c. blood of English heifer 430.

No result, and 420 was accordingly reinjected on the 31st January, 1907, subcutaneously with 50 c.c. of heifer 430. Temperature remained quite normal, and daily examinations of the blood failed to reveal any piroplasms.

Tested on immunity on 31st January, 1907, by subcutaneous injection of 10 c.c. blood of heifer 425, an animal immune to ordinary redwater.

Typical ordinary redwater reaction from 5th day, but piroplasma bigeminum not present. Secondary reaction from 17th day, lasting for about two weeks, and during which time piroplasma bigeminum and the lesions of anæmia appeared.

"*H*," *Heifer* 423.—A two-year-old heifer from Aliwal North, and susceptible to ordinary redwater.

Injected on the 13th December, 1906, intrajugularly with 5 c.c. blood of heifer 430.

As this injection failed to produce any results 423 received a subcutaneous injection of 50 c.c. from heifer 430 on the 3rd January, 1907.

With the exception of a sharp rise to 104.2 eight days later the temperature remained normal, and no piroplasms were seen in the blood preparations.

Tested on immunity on 30th January, 1907, by a subcutaneous injection of 10 c.c. blood of heifer 426, immune to ordinary

redwater. Reaction from the 6th day, piroplasma bigeminum appearing three days later.

"*I*," *Heifer* 424.—A two-year-old from Aliwal North, and therefore susceptible to ordinary redwater.

Injected on the 13th December, 1906, subcutaneously with 10 c.c. defibrinated blood of heifer 430. Temperature remained normal, and all blood examinations were negative. Accordingly 424 received a subcutaneous injection of 50 c.c. blood of heifer 430 on the 3rd January, 1907.

No result; the temperature remained normal, and no piroplasms were seen in the blood preparations.

Tested on immunity on 30th January, 1907, by subcutaneous injection of 10 c.c. blood of heifer 426, an animal containing piroplasma bigeminum in its blood.

Typical ordinary redwater from the 6th day, but piroplasma bigeminum not seen, although poikilocytosis and polychromatic cells were present.

Heifer 424 was again injected on the 8th March, 1907, with blood containing piroplasma bigeminum and piroplasma mutans from heifer 409.

No reaction, piroplasma mutans only appearing.

Notes on heifers 400, 418, 421, 422, 401, 419, 420, 423 *and* 424.

With regard to these nine heifers, two injections of English redwater blood failed in every instance to cause a reaction, and piroplasms did not appear.

A subsequent inoculation of South African redwater caused these injected heifers to contract this disease, and, with the exception of Nos. 400 and 424, piroplasma bigeminum appeared in every animal.

Heifers 400 and 424 were reinjected with blood containing piroplasma bigeminum, the result being that no reaction ensued, nor were piroplasms present.

Conclusions.

I do not feel justified in drawing any conclusions from the results of the injection of heifers 400, 418, 421 and 422 with blood of English heifer No. 428, for, as will be seen from Experiment 1, "*A*," this heifer did not appear to contract the infection in England, as no piroplasms were seen, due to an inoculation of English redwater blood, and the reaction was atypical. I therefore consider that the failure of heifer 428 to contract English redwater from the injection of virulent blood is another point in favour of my contention that English redwater is not always inoculable. The results obtained from the other five heifers, however, seem to be conclusive, as heifer 430 was certainly infected with English redwater.

Therefore English redwater was not inoculable in our five South African heifers, and accordingly when tested they all contracted ordinary redwater, proving that they had not acquired any immunity against South African redwater.

Exposure Experiments with the Imported Heifers.

Continuing on the lines of the arrangement made between Mr. Stockman and myself, the imported Ayrshire heifers were exposed on

the farm "Linwood," near Pretoria. The temperatures were taken daily, and the blood examined from time to time.

Heifer No. 428.—Exposed on the 5th January, 1907. Three days after the temperature commenced to rise, reaching 106, and constantly remaining high during the next 47 days. Nothing particular was noticed in the blood at the beginning of this reaction, but on the 35th day piroplasma bigeminum was noticed, remaining for some days, but disappeared from the 39th day. The lesions of poikilocytosis were occasionally noted, and the temperature returned to about normal on the 26th February. A second rise ensued on the 4th March, piroplasma bigeminum not being noticed, but poikilocytosis and marginal points appeared, and the animal remained very weak. Death occurred on the 17th March, with all the lesions of the sequel of ordinary redwater. The anæmia was so pronounced that the blood consisted almost entirely of basophile, polychromatic and nucleated cells.

Heifer No. 430.—Exposed at Linwood on the 5th January, 1907. Temperature commenced to rise on the 12th January, and then oscillated very irregularly for the next month; microscopical examination of the blood at repeated intervals failed to reveal piroplasma bigeminum, but the lesions of poikilocytosis were noted.

Recovered.

Heifer No. 429.—Exposed at Linwood on the 5th January, 1907. Reaction commenced six days after exposure, when the temperature rose to over 106, and remained high for the following 14 days. Spirillum, basophile cells, the lesions of poikilocytosis and marginal points were noted, but piroplasma bigeminum did not appear.

Recovered.

Heifer No. 432.—Exposed at Linwood on the 5th January, 1907. Irregular temperature noted soon after, and rose about three weeks later to a high elevation, touching 105.8; piroplasma bigeminum, the lesions of poikilocytosis, basophile granulations and polychromatic cells were present.

Recovered.

Heifer No. 431.—Exposed at Linwood on the 5th January, 1907. This animal also showed an irregular high temperature, reaching over 105, and as the maximum recorded 106. Poikilocytosis, basophile granulations and spirillum were noted. Piroplasma bigeminum was not present.

Recovered.

Heifer No. 433.—Exposed at Linwood on the 5th January, 1907. Irregular temperature noted on the 12th January, commencing with 106, maintaining high for the next 16 days, and touching 106.8 on the 31st January, 1907. Basophile granulations, polychromatic cells and poikilocytosis, accompanied with marginal points, were noted as the alteration in the blood. Piroplasma bigeminum was not noted, but the lesions of anæmia increased, and the animal died as the sequel of ordinary redwater.

RÉSUMÉ.

Heifer No.	Particulars.	Results when exposed to natural infection of Ordinary Redwater.
428	Not immune to English redwater ...	Reaction with piroplasma bigeminum ; died of ordinary redwater.
430	Immune to English redwater ...	Reaction : recovered.
429	Immune to ordinary redwater ...	Reaction ; recovered.
432	Immune to ordinary redwater ...	Reaction with piroplasma bigeminum : recovered.
431	Immune to ordinary redwater, but susceptible to English redwater	Reaction ; recovered.
433	Immune to both English and South African redwater	Reaction : died of sequel of ordinary redwater.

As already stated, I am of the opinion that English redwater does not protect against South African redwater, and the above statement furnishes additional support to this conception. In every case a new reinfection occurred, in two instances accompanied with piroplasma bigeminum.

"C."—FURTHER TRANSMISSION EXPERIMENTS WITH EAST COAST FEVER.

In my Annual Report for 1903-4, I enumerated various experiments, the conclusions of which were as follows:—

Rhipicephalus decoloratus (the common blue tick) is not a host of piroplasma parvum.

Rhipicephalus evertsi (the red tick) is not a host of piroplasma parvum.

Rhipicephalus simus is a host of piroplasma parvum.

Amblyomma hebræum may be a host of piroplasma parvum.

Rhipicephalus appendiculatus (the brown tick) is the principal host of piroplasma parvum, and it was further stated that brown ticks transmit the disease principally in their imago stage, after having fed as nymphæ on sick beasts; less so as nymphæ, after having fed as larvæ, and not at all as larvæ originating from a mother tick removed from a beast infected with East Coast fever. In other words, piroplasma parvum does not pass from the female imago into the egg and from this into the larvæ, as is the case in Texas fever. These experiments were carried out almost simultaneously with those of Mr. Lounsbury, of Capetown, and as a result the facts were established that the blue tick under no conditions acts as a host of piroplasma parvum, and that the brown tick (the tick with three hosts) is the principal carrier of the disease.

In 1906 Mr. Lounsbury published a further series of experiments which proved that, besides the ticks mentioned, others transmit the disease, viz., rhipicephalus nitens, evertsi and capensis. In *Mense's Handbuch der Tropenkrank-heiten,* 1906, a contribution appeared by Luhe regarding the protozoa parasite in the blood, and in regard to my statement that "piroplasma parvum does not pass through the egg," he makes the objection that in my experiments the larvæ which were employed were too young, and he quotes a communication of Professor Koch, who exposed larval ticks hatched in the laboratory.

on a pasture, and thus created a new herd of infection. Professor Schilling in his contribution on piroplasmosis in the *Handbuch der Pathogenen Mikroorganismen* said that Gray and Robertson had already expected that rh. decoloratus (blue tick) had to be considered as a carrier of the disease. From former experiments Koch knew that the German East African Coast fever which had proved to be identical with the Beira Coast fever was transmitted by ticks. Koch took female ticks which had repleted themselves on sick cattle and placed them in a warm and humid atmosphere for the laying of the eggs; these larvæ were placed on a pasture on which hitherto only a few animals had become infected with East Coast fever. The young ticks did not leave the pasture, but were waiting on the tops of the grasses until the cattle passed by in order to attach themselves. Soon after, and on this very pasture, grave cases of East Coast fever began to appear, and it was possible to infect every fresh susceptible animal which was liberated on this place.

It is apparent from the above communication that the opinions of Mr. Lounsbury and myself (who do not consider the blue tick as a carrier of the disease) are the reverse to those of Professor Koch. In Koch's experiment, as cited by Schilling, he speaks of ticks in general, but at the Inter-Colonial Veterinary Conference, held at Bloemfontein in 1903, Koch, in referring to this particular experiment, speaks exclusively of the blue tick.

It must be stated here that the experiment as carried out by Professor Koch does not withstand strong criticism. It was performed by placing young larval ticks on a pasture on which hitherto only a few animals had become infected, that is to say, on an East Coast fever infected pasture. Accordingly, with the introduction of fresh susceptible cattle the spread of the disease had naturally to be expected, and would have taken place even if the new ticks had not been liberated. Indeed it is probable that between the first and second exposure of susceptible cattle, the larval and nymphal ticks already present had moulted, and thus were responsible for the increase of the disease.

With regard to my statement that an animal immune against East Coast fever does not act as a propagator of this disease, Professor Schilling says: "In his last report Theiler's experiments would prove that salted cattle do not infect the ticks (which species?). This is directly contradictory to the history of the introduction of the disease into Rhodesia. From which cattle did the animals imported from New South Wales contract the infection, if not by means of ticks from the herds grazing in the neighbourhood of Beira, amongst which, as Koch has proved, carriers of parasites were constantly present."

The results of my experiments proving that salted cattle do not transmit the infection were published in my Annual Report for the year 1904-5, and also in the *Journal of Comparative Pathology and Therapeutics* in the year 1905, under the title, "Do salted cattle contain piroplasma parvum in their blood?" In this article it was proved that the brown tick (rh. appendiculatus) neither in the nymphal nor the imago stage is capable of transmitting East Coast fever after having fed as larvæ or nymphæ on an immune animal. These results were corroborated by Lounsbury in his investigations published in 1905-6 under the title, "Ticks and African Coast Fever." Lounsbury experimented 16 times on nine different animals with ticks, of which

he was sure that they were acting as hosts of piroplasma parvum, viz.:—Rh. appendiculatus in their nymphal and imago stage, with rh. nitens and evertsi in their imago stage, and in no case could he transmit the disease from the immune animal to the susceptible one. Convincing proof can be found in practice, since it is frequently noted that immune animals have been grazing with susceptible ones for years on one and the same pasture, and yet the latter have never contracted the disease.

We are in possession of ten immune oxen which in 1902 were the survivors of a herd of 500 head destroyed by the disease. These ten oxen have been repeatedly exposed on an infected area, thereby proving their immunity. For over four years they constantly grazed with a herd of about 50 susceptible animals on a non-infected pasture, on which brown and red ticks were present, but hitherto no disease has been noted. This experiment was purposely continued for this length of time in order to exclude every possibility of coincidences. Therefore the occurrence in Beira can only be interpreted in a different manner. From Mr. Gray's report to the Rhodesian Government, it clearly follows that the Australian cattle imported into Beira were only suffering from redwater (Texas fever), and this was the reason for bringing them to the higher town of Umtali, in which case the extraordinary appearance of East Coast fever was noted. The cattle grazing in Beira had nothing to do with the infection with the cattle in Umtali. Indeed it has to be accepted that at least at that time Beira was not infected with East Coast fever, and in proof of this it must be mentioned that Madagascar oxen which were imported into Beira almost simultaneously with the Australian herd never suffered or died of East Coast fever, and not even after the Australian cattle had been removed to Umtali. Madagascar cattle are equally susceptible to East Coast fever as Australian cattle, but the former are immune to ordinary redwater, whereas the latter are not.

From the communication of Mr. Orpen, which I have detailed on page 9, it is clear that Beira was infected with redwater, and later it appeared that slaughter cattle from East Africa were imported direct from Umtali, and probably brought East Coast fever with them.

The following experiments were partially carried out for the purpose of corroborating my former communications; some of the experiments of Lounsbury were repeated, and the opinion of Luhe was considered as correct, and accordingly taken into consideration. The ticks for these experiments were collected on the coast at Durban from cattle which were all visibly suffering from East Coast fever, the diagnosis being confirmed either by post-mortem or by microscopical examination.

Experiment No. 1.

To prove that ticks collected on the coast of Natal from sick animals are capable of transmitting East Coast fever.

(a) *Experiments with brown imagines.*

N.B.—The brown nymphæ used for infesting the following animals were all collected on the 16th December, 1906, in Durban.

"*A*," *Ox* 358.—Two years old; from Cape Colony. Infested with 12 brown imagines about 20 days old on the 17th January, 1907.

On the 28th January a fever reaction started, and on the following day piroplasma parvum was present, daily increasing in numbers until the 10th of February, on which day the animal died of East Coast fever.

Post-mortem Examination.

Condition:—Fair. Rigor mortis present. Beef somewhat pale. Lymphatic glands in groin and in front of shoulder enlarged.

Lungs:—Oedematous; considerable amount of yellow liquid in pleural cavity; yellow liquid in intersepta; mediastinal tissue infiltrated; glands also infiltrated.

Heart:—A few hæmorrhagic patches on epicard and in left ventricle.

Spleen:—Normal.

Liver:—Thicker than normal; section yellowish; gall bladder small; bile yellow and viscid; lymphatic glands enlarged.

Kidneys:—Calix infiltrated; small white infarcts about the size of a pin's head and a few petechiæ on surface.

Stomach:—Fourth mucosa pale, and containing a few hæmorrhagic ulcers the size of a pea. Contents of third stomach soft.

Intestines:—Cæcum and colon mucosa pale; black patch on ileo-cæcal valve; mucosa of small intestines partially black.

"*B*," *Ox* 387.—From the Cape Colony. One year old.

Infested on the 1st March, 1907, with nine brown male imagines, about 27 days old.

Temperature commenced to rise on the 11th March, and piroplasma parvum was noted six days later, daily increasing in numbers until the death of the ox on the 26th March, 1907.

Post-mortem Examination.

Condition:—Poor. Rigor mortis set in; lymphatic glands of left shoulder enlarged; beef normal.

Lungs:—Slightly œdematous.

Heart:—Yellow liquid in peritoneal cavity; left endocard echymosed; a few hæmorrhages on right endocard.

Spleen:—Enlarged and congested.

Liver:—Enormously enlarged, soft, and of a peculiar red colour.

Kidneys:—Congested, and containing a few white infarcts. Urine clear.

Bladder:—Gall bladder filled with yellow bile.

Stomach:—Mucosa diffusely congested and swollen; contents dry.

Intestines:—Diffiuse congestion of cæcum and colon; small intestines diffusely hæmorrhagic.

Lymphatic glands of liver:—Considerably enlarged.

"*C*," *Bull* 327.—Two years old; animal from Potchefstroom, a district hitherto free from East Coast fever.

Infested on the 4th April, 1907, with brown imagines about 62 days old, and in the morning of the 15th April the temperature commenced to rise. The disease lasted until the 24th day after the infestation, on which date the animal died. Piroplasma parvum was noted daily in the blood from the 19th April up to the time of death, on which date piroplasma bigeminum also appeared.

Post-mortem Examination.

Condition:—Rigor mortis not complete; blood of a brownish hue and not completely coagulated; serous membranes and fasciæ of a yellowish colour; mediastinal glands infiltrated with gelatinous liquid.

Lungs:—Slight œdema.

Heart:—Both endocards normal.

Spleen:—Enlarged and soft.

Liver:—Enlarged and thick; contained yellow spots.

Kidneys:—A few white infarcts; yellow spots.

Bladder:—Thick bile of a greenish colour in gall bladder.

Stomach:—Folds of mucosa infiltrated and thickened; omasus filled with dry food.

Intestines:—Mucosa of small intestines swollen and bile stained. Longitudinal slate-coloured stripes on colon and cæcum.

"*D*," *Heifer* 416.—Born on the station; about one year old.

Infested on the 19th March with male and female imagines about 45 days old. After an incubation time of about 10 days the temperature started to rise, culminating in the death of the animal on the 13th April, 1907. Piroplasma parvum noted daily from the 3rd day after rise of temperature.

Post-mortem Examination.

Condition:—Fair; peritoneal cavity contained non-coagulated blood.

Lungs:—Oedematous; foam in trachea.

Heart:—Left endocard normal; white spots in tissue.

Kidneys:—Contained red and white infarcts; capsula infiltrated with blood.

Bladder:—Gall bladder contained bile of a thick and dark greenish colour.

Stomach:—Congestion of mucosa of fourth stomach, with a few hæmorrhagic ulcers.

Intestines:—Several hæmorrhagic ulcers, about the size of a pea, distributed over cæcum. Mucosa of small intestines swollen and congested.

Glands:—Lymphatic glands slightly enlarged.

"*E*," *Ox* 391.—A Cape Colony animal; aged.

Infested on the 23rd April, 1907, with male and female brown imagines, 108 days old.

After an incubation time of 11 days the temperature of this animal rose, and the disease lasted eight days.

Piroplasma parvum noted daily from the 3rd day after reaction, the animal dying of East Coast fever on the 12th May, 1907.

Post-mortem Examination.

Condition:—Very poor; rigor mortis not set in; beef pale; gelatinous infiltration of mediastinal glands.

Lungs:—Slight œdema; one echymose on left side of costal pleura; pleural cavity contained blood-stained liquid; fibrous adhesions on sternalis.

Heart:—Diastole; blood well coagulated; gelatinous infiltration of left endocard; imbibition in right endocard; pericard full of liquid; epicard injected.

Spleen:—Normal.
Liver:—Slightly enlarged; commencement of decomposition.
Kidneys:—Pale; gelatinous infiltration of calix.
Bladders:—Urinary bladder filled with normal urine. Gall bladder distended; contained thick bile.
Stomach:—Mucosa of abomasus partially slate and red coloured; omasus normal.
Intestines:—Cæcum contained reddened patches, with injection of blood vessels. Bluish colour on outside of small intestines; mesentery slightly infiltrated; mucosa diffusely reddened with superficial erosions; colon normal.

Experiment No. 2.

With larvae of blue ticks which were collected at the same time and from the same animals as the ticks used for the previous experiments.

In the previous experiments with imagines of rh. appendiculatus absolute proof was given that these brown ticks from the Natal cattle were capable of communicating East Coast fever. If the blue tick is a host of piroplasma parvum it must be granted that blue ticks originating from the same animals which supplied us with the pathogenic brown ticks must also transmit the disease, especially if they were placed on animals in large numbers.

N.B.—The larvæ utilised for the infestation of the following animals, hatched from the 23rd March, 1907, onwards.

"*A*," *Heifer* 421.—A two-year-old, from Aliwal North. Heavily infested with blue tick larvæ, about 12 days old, on the 4th April, 1907. From the 29th April the dropping engorged females were collected in great numbers. This heifer did not sicken, and is still alive.

"*B*," *Heifer* 426.—Two years old, from Aliwal North. Infested with blue larval ticks, about 12 days old, on 4th April, 1907.
No result; the animal is still alive.

"*C*," *Heifer* 400.—Aliwal North, two years old. Infested with larval blue ticks on the 24th May, 1907, two months after they had hatched. The repleted females dropped from the 19th June, 1907, in large numbers. No disease ensued; the animal is still amongst our herd.

"*D*," *Calf* 440.—Born on the station, about six months old. Infested with blue larval ticks, about 60 days old, on 24th May, 1907. No result; the animal was utilised on the 1st August, 1907, for experiments in connection with pleuro-pneumonia, and died as a result of the inoculation on the 26th August, 1907.

Experiment No. 3.

With blue tick larvae originating from an ox immune against East Coast fever.

"*A*," *Ox* 377.—An animal from Sjamboks Kraal, Pretoria District, and one of a survivor of a herd consisting of about 200 which were all destroyed by East Coast fever. The diagnosis in this animal was made by proving the presence of piroplasma parvum during the disease. On the 9th March, 1907, ox 377 was

purchased and brought to the station, and is still here at the present time. At repeated intervals 377 was infested with blue ticks, and the brood of larval ticks of the last lot collected were utilised for the succeeding experiments.

In order to prove that this ox was immune to East Coast fever it was infested with brown imagines on the 4th April, 1906. These brown imagines belong to the same collection as those which produced the disease on animals 416, 327 and 391. No signs of illness were noted, and hence further proof is given of the immunity of this ox.

N.B.—The larvæ used for the infestation of the following animals hatched on the 5th November, 1906.

"*B*," *Heifer* 413.—Two years old, from Pretoria, infested on the 5th December, 1906, with blue tick larvæ 31 days old. Seven days after infestation a rise of temperature was noted for seven days, followed by a second rise running for a longer period, during which spirillum were noticed for several days. The repleted females dropped from the animal on the 23rd December, 1906, and were collected in great numbers. In order to make sure whether the reaction had any connection with East Coast fever, it was decided to test this animal on its immunity. Accordingly 413 was infested on the 16th April, 1907, with nymphal brown ticks which had repleted themselves on animal 387 during the time it was suffering from East Coast fever. From the 22nd April, 1907, onwards, the dropping nymphæ were collected. On the 27th April, 1907, fever appeared in animal 413; piroplasma parvum was noticed from the 2nd to the 9th May, on which latter date piroplasma bigeminum was seen, and the animal died on the 11th May.

Post-mortem Examination.

Condition:—Fair; rigor mortis not completely set in; lymphatic glands of the groin and serous membranes pale and swollen; blood not coagulated, but of a brownish hue.

Lungs:—Rather pale.

Heart:—Diastole; right endocard normal; left endocard echymosed.

Spleen:—Slightly enlarged.

Liver:—Swollen; section of a peculiarly glossy appearance.

Gall Bladder:—Contracted and containing liquid bile.

Kidneys:—Contained a few infarcts; slight gelatinous infiltration of calix. Urinary bladder contracted.

Stomach:—Normal.

Intestines:—Mucosa of small intestines slightly swollen. Cæcum normal.

"*C*," *Ox* 359.—Two years old, from Aliwal North. Infested on the 5th December, 1906, with blue larval ticks, about 31 days old. In this animal a slight reaction was noticed, the nature of which was recognised. 359 was an animal used in a former experiment in connection with piroplasma mutans, and had been injected on the 24th August, 1906, frequently showing piroplasma mutans at repeated intervals from the 25th September, 1906, so that the reappearance of these parasites during the time of the tick infection must be considered as a coincidence, but the very fact that Professor Koch identified these ring-shaped parasites with piroplasma parvum induced me to test this animal on its

immunity to East Coast fever. Four brown females originating from Durban were placed on 359 on 13th February, 1907, and ten days later a rise of temperature was noticed. On the 2nd March, 1907, piroplasma parvum was seen, increasing rapidly until seven days later, when the animal died of East Coast fever.

Post-mortem Examination.

Condition:—Rather poor; rigor mortis not completely set in. Beef pale; mediastinal lymphatic glands greatly enlarged.

Lungs:—Normal.

Heart:—Normal.

Spleen:—Enlarged; pulpa softened.

Liver:—Abnormal and of a bluish brown colour, with numerous white spots.

Kidneys:—Full of red and white infarcts.

Bladder:—Dark brown bile in gall bladder.

Stomach:—Third stomach normal.

Intestines:—Diffuse hæmorrhagic infiltration of small intestines, and containing a few hæmorrhagic small ulcers. Hæmorrhagic infiltration of cæcum.

Experiment No. 4.

To transmit East Coast fever with red ticks (rhipicephalus evertsi).

As already stated, my previous experiment with red ticks was negative. This was carried out by placing numerous red ticks on one and the same animal without the disease being noticed, but, as only one animal was utilised, the objection may be raised that, by a coincidence, the animal was immune.

"A," *Ox* 357.—Two-year-old animal, from Aliwal North. Infested on the 23rd April, 1906, with red imagines which were collected from animals suffering from East Coast fever at Sjambok's kraal. A further infestation took place on 25th and 26th April, and, commencing on 5th May, the repleted females dropped, and a rise of temperature began five days later. On 15th May piroplasma parvum was noticed for the first time, increasing rapidly until 29th May, on which day the animal died.

Post-mortem Examination.

Condition:—Fair; some foam in nostrils; subcutaneous tissue œdematous.

Lungs:—Some petechiæ on pleura in region of heart, mediastinum œdematous; some clear fluid in pleural cavity; mediastinal gland congested; foam in trachea.

Heart:—Punctiform hæmorrhages on pericardium, which also contained some blood-tinged liquid; myocardium normal; epicardium shows some blood-staining (probably post-mortem).

Spleen:—Normal.

Liver:—Slightly icteric; hepatic glands congested; gall bladder normal; bile rather thick and yellow.

Kidneys:—Peri-renal fat hæmorrhagically infiltrated; the whole of the cortices covered with hæmorrhages, which also extended into medulla boundary layer and calix.

Bladder:—Urine quite clear.

Stomach:—Fourth stomach slightly congested; contained a few petechiæ.

Intestines:—General congestion, varying from slight to intense.

"*B*," *Cow* 455.—Originating from Pretoria. Infested on 23rd May, 1907, with red imagines, 67 days old, which, in their larval and nymphal stage, had been feeding on ox 358 whilst it was suffering from East Coast fever, and which had moulted on 17th March. After an incubation time of 14 days fever appeared. The reaction lasted 13 days, and the animal died on the 19th June, 1907.

Piroplasma parvum noted four days after the beginning of the reaction, and increased daily.

Post-mortem Examination.

Condition:—Fair; abscess between liver and right kidney.

Lungs:—Normal; right lobe swollen; fibrous induration adjoining abscess capsula.

Heart:—Pericard normal; sugillations in left ventricle and white patches in right ventricle.

Spleen:—Very slightly enlarged.

Liver:—Swollen; friable; yellow patchy discolouration.

Kidneys:—Capsula of right kidney enlarged and containing cheesy matter. White infarcts.

Stomach:—Abomasus congested, and containing a few small ulcers the size of a pea.

Intestines:—Cæcum disseminated with ulcers the size of a pea, and which had a red circumference. Colon also contained similar ulcers; mucosa swollen with red patches. Mucosa of small intestines swollen, and covered with ulcers similar to those in cæcum.

"*C*," *Heifer* 45.—Infested in London by Stockman on the 25th June, 1906, with the same brood of ticks utilised in the first experiment, a number of which were sent to Mr. Stockman, Principal Veterinary Surgeon of England, in order to control the appearance or otherwise of piroplasma parvum in cattle outside South Africa, and especially to show whether piroplasma parvum would develop in cattle that never have been in contact with South African animals. Commencing on the 8th July, 1906, the animal showed a typical reaction, and died on the 10th July, 1906, on which date piroplasma parvum was noticed in almost every blood corpuscle. Stockman was good enough to forward me blood preparations for examination, and the correctness of the diagnosis was indisputable.

Experiment No. 5.

With amblyomma hebræum (the bont tick).

These ticks were also collected from cattle on the coast of Natal. It is known that this specie belongs to the ticks with three hosts. Hitherto we have found that (*a*) ticks with three hosts are capable of transmitting the disease as nymphæ after having fed as larvæ on sick animals; (*b*) as imagines after having fed as nymphæ on sick cattle; and (*c*) it has naturally to be expected that the infection passes through the egg.

(A) *Imagines of amblyomma hebræum.*

"*A*," *Ox* 391.—A full-grown ox, from Klipplaats, Cape Colony. Infested on the 4th March, 1907, with imagines, about 78 days old, collected on the 16th December, 1906, from sick cattle on the Durban coast. Reinfested on the 5th March, 1907, and the

following day male and female ticks were found fast. The engorged females dropped on the 30th March, 1907; no reaction was noted. Ox 391 was tested later on its immunity against East Coast fever, and died (compare Experiment 1, "*E*").

"*B*," *Ox* 389.—Full grown, from Klipplaats, Cape Colony. Infested on the 26th March with imagines, about 100 days old, from the same collection as the previous case. The repleted females were collected from the 7th April, 1907. No reaction ensued. This ox was tested on its immunity on the 6th May, 1907, with nymphæ which as larvæ had fed on ox 387 during its illness. A rise of temperature was noted on the 18th May, and a typical fever reaction followed. Piroplasma parvum was noted on the 25th May, 1907, and was very frequent five days later. In order to obtain blood for the purpose of hyperimmunisation this animal was bled to death on the 30th May, 1907.

Post-mortem Examination.

Condition:—Poor; flesh pale; fasciæ and omentum yellow.

Lungs:—In inspirium; slight œdema; some yellow liquid in peritoneum.

Heart:—Endocard normal.

Spleen:—Very slight congestion.

Liver:—Enlarged; brown.

Kidneys:—Slight gelatinous infiltration of calix; a few white areas the size of a pin's head.

Stomach:—Abomasus pale.

Intestines:—Cæcum and small intestines normal.

(B) *Larvæ of amblyomma hebræum.*

These originated from female ticks which were collected on the 18th December, 1906, in Durban. The eggs had been laid on the 27th December, 1906, and had hatched on the 18th April, 1907.

"*C*," *Heifer* 418.—A two-year-old, from Aliwal North. Infested on the 24th and 25th May, 1907, with the above larvæ, which on this day were 36 days old. No reaction noted, and the animal is still alive.

"*D*," *Heifer* 419.—A two-year-old, from Aliwal North. Infested on the same date, and with the same brood of larvæ, about 36 days old. No reaction; the animal is still alive.

Experiment No. 6.

With rhipicephalus capensis.

As already stated, Mr. Lounsbury proved that these ticks, which are especially frequent in the south-eastern parts of Cape Colony, are capable of transmitting East Coast fever. This is also a tick with three hosts. Lounsbury sent me infected imagines which were utilised to verify his results.

"*A*," *Heifer* 379.—Originating from Capetown. Infested on the 15th June, 1906, with the above imagines. After an incubation period of 30 days the disease was noticed. The animal died on the 43rd day after infestation, *i.e.*, 28th July, 1906. Piroplasma parvum was noticed on the 20th July for the first time, and increased daily. On the day previous to death piroplasma bigeminum was also seen.

Post-mortem Examination.

Condition:—Very poor; evidence of diarrhœa; foam in nostrils.
Lungs:—Right lung adherent to chest wall (of old standing).
Heart:—Normal.
Spleen:—Normal.
Liver:—Normal.
Kidneys:—One infarct in right kidney.
Stomach:—Abomasum slightly congested.
Intestines:—Slight general congestion.

"*B*," *Heifer* 383.—Originated from Capetown. Was kept in the same stable as the previous animal, but not infected with imagines, notwithstanding which East Coast fever appeared. The disease could only have been transmitted by the ticks from heifer 379, inasmuch as at that time no experiments were carried out with any other species of ticks; this is the sole accidental infection that has occurred on our station. The animal died on the 30th July, 1906.

Post-mortem Examination.

Condition:—Poor.
Lungs:—Normal.
Heart:—Normal.
Spleen: Enlarged; pulp dark.
Liver:—Ochre coloured.
Kidneys:—Numerous infarcts in both kidneys.
Bladder:—Urinary bladder distended with clear blood-coloured urine.
Stomach:—Folds of abomasum œdematous.
Intestines:—Moderate general congestion.

Experiment No. 7.

Transmission of East Coast fever with progeny of infected brown ticks which as imagines were collected on sick animals in Natal.

I have already proved that rhipicephalus appendiculatus is the principal host of piroplasma parvum, and that the transmission in no case takes place via the egg, but in the nymphal or imago stages. However, should a transmission through the larval stage be possible, it has to be expected that this species would in the first instance be capable of so doing. The following experiments were accordingly made:—

"*A*," *Heifer* 386.—From Capetown; two years old. Infested on the 13th February, 1907, with brown larvæ whose mothers had been collected on the 16th December, 1906, in Durban. The females had started to lay eggs on the 20th December, 1906, which hatched on the 23rd January, 1907, thus at the date of the infestation the larvæ were 21 days old. On the 15th February, 1907, they were fast: they commenced to replete on the following day, and on the 22nd February, 1907, the first dropping engorged larvæ were collected. Subsequently several infestations were made with the same brood of larvæ up to the 20th February, 1907. The dropping larvæ were collected until the 10th March, 1907, in great numbers. No reaction was noted: this animal died later from inflammation of the intestines.

"*B*," *Heifer* 395.—Two and a half years old, from Aliwal North. Infested at the same time and with the same brood of ticks as in the former case. No reaction noted, and the animal is still alive.

"*C*," *Heifer* 398.—Two years old, from Aliwal North. Infested in the same way as above. No reaction.

"*D*," *Heifer* 402.—Two years old, from Aliwal North. Infested as above. No reaction.

"*E*," *Heifer* 408.—Two years old, from Aliwal North. Infested on the 12th March, 1907, with larval ticks from the brood used in the above experiments, which had hatched since the 23rd January, 1907; thus on the date of infestation were 48 days old. On the 15th March, 1907, brown engorged larvæ dropped, and on the same date the animal was infested with a fresh brood of brown larvæ whose mothers were collected on the 18th December in Durban, and which had hatched since 23rd January, 1907—thus were 49 days old. Engorged larvæ from 408 were collected in great numbers. No reaction.

"*F*," *Heifer* 409.—Two-and-a-half-year-old animal; infested for the first time on the 12th March, 1907, with larval ticks of the same brood as in the former experiment, and on the 22nd March, 1907, was infested for the second time with brood of larvæ which had hatched on the 25th January, 1907, and accordingly were 56 days old. Engorged larvæ were collected from the 26th March, 1907, onwards. No reaction. The animal met with an accident on the 8th July at Onderstepoort, and had to be killed.

"*G*," *Heifer* 412.—Two years old, from Aliwal North. Infested on the 12th March, 1907, with brown tick larvæ which had hatched on the 23rd January, 1907, and the engorged larvæ were collected from the 16th March, 1907. Reinfested on the same date with larvæ hatched on 25th January, 1907—50 days old. Engorged larvæ collected from the 19th March onwards. A third infestation with larvæ of the same origin took place on the 22nd March, 1907, and the collection of the engorged larvæ commenced three days later. No reaction, and the animal is still alive.

"*H*," *Heifer* 420.—Two years old, from Aliwal North. Infested on the 12th March, 1907, with brown larvæ which had hatched on 23rd January, 1907. A second infestation on the 16th March with larvæ hatched on the 25th January, 1907. A third infestation with larvæ of the same origin on the 22nd March, 1907. No reaction.

"*H*," *Heifer* 422.—Two years old, from Aliwal North. Infested on the 12th March with brown larvæ originating from mother ticks collected on the 16th December, 1906, in Durban, and which hatched since 23rd January, 1907—accordingly were 48 days old. On the 16th March, 1907, a second infestation with larvæ which had hatched on the 25th January. No reaction, and the animal is still alive.

"*J*," *Heifer* 401.—Two-year-old, from Aliwal North. Infested on the 13th, 16th and 19th March, 1907, with larvæ whose mothers

had been collected on the 18th December in Durban, and which had hatched since 23rd January, 1907. No reaction, and the animal is still alive.

"*K*," *Heifer* 404.—Two years old, from Aliwal North. Infested on the 13th, 19th and 22nd March, 1907, with larvae of the same brood as utilised above. No reaction, and the animal is still alive.

"*L*," *Heifer* 407.—Two years old, from Aliwal North. Infested on the 19th and 22nd March with the same larvæ as before. No reaction, and the animal is still alive.

"*M*," *Heifer* 453.—From Klipplaats, Cape Colony. Infested on the 10th April, 1907, with brown larval ticks originating from mothers collected on the 14th February, 1907, in Durban, and which had hatched since the 23rd March, 1907. No reaction. The animal is still alive.

"*N*," *Heifer* 454.—A two-year-old animal, from Aliwal North. Infested on the 19th April, 1907, with brown larval ticks whose mothers were collected on the 29th January, 1907, in Durban, and which had hatched on the 24th February, 1907—accordingly were 54 days old. No reaction. A subsequent inoculation with blood from an animal immune against redwater caused 454 to contract this disease, and it died on the 20th July, 1907.

"*O*," *Heifer* 419.—From Aliwal North, and about two years old. Infested on the 2nd April, 1907, with the same brood of ticks as used above, the larvæ accordingly being 65 days old. No reaction.

"*P*," *Heifer* 449.—A two-year-old, from Aliwal North. Infested on the 2nd April, 1907, as above. No reaction.

"*Q*," *Heifer* 418.—About two years old, and imported from Aliwal North. Infested on the 2nd April, 1907, with larvæ of the same brood as above, and which were 71 days old. No reaction.

"*R*," *Heifer* 445.—About two years old, and imported from Aliwal North. Infested as above. No reaction.

"*S*," *Heifer* 452.—About two years old, from Klipplaats, Cape Colony. Infested on the 10th April, 1907, with brown larvæ originating from mother ticks collected on the 23rd January, 1907, in Durban. Larvæ hatched from the 24th February, and, at date of infestation, were accordingly 45 days old. Reinfested with larvæ of the same origin on the 19th April. No reaction; the animal is still alive.

Experiment No. 8.

With nymphæ of the brown ticks whose mothers had been collected in Durban and which, as larvæ, had been utilised in the previous experiments.

The origin of the following animals is:—Heifers 435 and 439 came from England. 440 was born on the station. 449, 450 and 451 came from Klipplaats, and the remainder from Aliwal North.

On the 25th March, 1907, heifers 408, 409 and 422 were infested with brown nymphæ.

On the 27th March, 1907, 409 was reinfested.

On the 28th March, 1907, 422 was reinfested and 404, 407, 412 and 420 infested.

On the 30th March, 1907, full engorged nymphæ dropped from heifers 408 and 409 and 422, and on the 2nd April, 1907, from animals 404, 407, 412 and 420.

On the 4th April, 1907, heifers 450 and 451 were infested with brown nymphæ, and reinfested two days later.

On the same date 440 was infested, and was reinfested on the 9th April, 1907.

On the 10th April, 1907, heifers 394, 398 and 435 were infested.

On the 12th April, 1907, and with the same brood, 439 was reinfested.

On the 13th April, 1907, reinfested 450, 451, 394, 398 and 435, and freshly infested 395, 402 and 405.

On the 15th April, 1907, reinfested 440.

On the 17th April, 1907, reinfested 435 and 439.

On the 18th April, 1907, reinfested 450.

On the 20th April, 1907, reinfested 451.

On the 22nd April, 1907, reinfested 440.

On the 23rd April, 1907, reinfested 435, 439, 450, 451, and on the 26th April, 1907, 435 and 439.

On the 16th May, 1907, reinfested 418, 419 and 449.

None of these 18 animals showed a reaction consequent to the tick infestation, and, with the exception of 409 (*vide* Experiment 7, "*F*," killed on account of an accident) and 440 (*vide* Experiment 1, "*D*," died of pleuro-pneumonia) are all still alive.

Experiment No. 9.

With imagines, which, as larvæ and nymphæ, have been feeding on healthy animals; the mothers of these imagines originated from sick animals.

N.B.—The mothers were collected in Durban on the 16th December, 1906, from animals suffering from East Coast fever. The larvæ had been feeding on animals Nos. 386, 395, 398, 402, 408, 409, 412, 420, 422, 401, 404, 407 and the nymphæ on the animals Nos. 394, 395, 398, 402, 404, 405, 407, 408, 409, 412, 420 and 422, and were collected from the 23rd March, 1907, up to the 22nd April, 1907, from these animals. The engorged nymphæ moulted from the 5th to the 30th May, 1907, and on the 27th May, 1907, the imagines were placed on animals Nos. 446, 447, 450, 451, 452 and 454. On the 3rd June, 1907, all these heifers were reinfested with the same brood of imagines.

Heifer 446.—An irregular temperature reaction noted in this animal, and on the 7th June, 1907, piroplasma bigeminum was seen. The animal is still alive.

Heifer 447.—Nothing noted in this animal. On the 8th July, 1907, infested with brown nymphæ which as larvæ had fed on sick ox 387. From the 25th July, 1907, fever was noticed, and on the 10th August, 1907, this animal died of East Coast fever.

Post-mortem Examination. Made ½ hour after death.

Condition:—Fair; rigor mortis not yet set in; flesh somewhat pale.

Lungs:—In state of inspirium: œdematous; foam in trachea.

Heart:—Pericard normal; extensive hæmorrhages in left and right ventricles.

Spleen:—Normal.

Liver:—Swollen, hyperæmic and of a peculiar glossy appearance.

Kidneys:—Oedematous.

Bladder:—Gall bladder slightly contracted; contained yellow viscid bile.

Stomach:—A few superficial hæmorrhagic ulcers on mucosa of fourth stomach.

Intestines:—Several superficial hæmorrhagic ulcers and hæmorrhages in cæcum; mucosa of colon normal; small intestines presented a swollen appearance all through, with yellow contents adherent to mucosa; mucosa swollen, cross-striped and red.

Heifer 450.—Nothing particular noticed in this animal, which is still alive.

Heifer 451.—Irregular reaction on the 6th July, 1907. Piroplasma bigeminum noted. The animal is still alive.

Heifer 452.—Nothing particular noted in this animal. Still alive.

Heifer 454.—Nothing particular noted in this animal. Still alive.

Résumé.

1. The larvæ of the blue tick (rhipicephalus decoloratus) which originated from females feeding on (*a*) animals suffering from East Coast fever, and (*b*) from animals immune to East Coast fever, did not transmit the disease.

2. The larvæ of amblyomma hebræum, originating from mothers removed from sick cattle and of the imagines originating as nymphæ from sick cattle, did not transmit the disease.

3. All experiments to transmit the disease by means of the progeny (larvæ, nymphæ, and imagines) of brown ticks whose mothers had been feeding on sick animals had no results, notwithstanding the fact that the young ticks were kept for a considerable length of time before they were used for the experiments.

4. East Coast fever was transmitted (*a*) by the nymphæ of rhipicephalus appendiculatus which as larvæ had been feeding on

animals suffering from East Coast fever, (*b*) by imagines of rhipicephalus appendiculatus, evertsi, and capensis, all of which had infected themselves as nymphæ.

Conclusions.

Rhipicephalus decoloratus and amblyomma hebræum must not be considered as hosts of piroplasma parvum.

Rhipicephalus appendiculatus, evertsi, capensis, simus, and, according to Lounsbury, also nitens, must be considered to be hosts of piroplasma parvum. It may safely be concluded that piroplasma parvum in its life cycle of development does not pass through the egg, and finally it is evident that immune animals do not carry the infection.

"D."—RESULTS OF HORSE-SICKNESS INOCULATION IN PRACTICE DURING 1906-1907.

The inoculation of mules against horse-sickness was recommenced from July, 1906, by the respective Government Veterinary Surgeons of the following districts:—Barberton, Lydenburg, Pretoria, Johannesburg, Krugersdorp, Potchefstroom, Wakkerstroom (Piet Retief and Volksrust), Zoutpansberg, Marico, Rustenburg, Waterberg, Ermelo, Standerton and Heidelberg. The immunisation was also performed in Rhodesia, Natal, Orange River Colony and Bechuanaland Protectorate.

Up to June, 1906, the virus and serum utilised was known as the "Ordinary" strain, but in experimenting I found that a virus from Tzaneen was able to break the immunity conferred by the Ordinary strain, and accordingly it was introduced into practice, as I considered it would afford a better protection. This Tzaneen strain gave good results until December, when from reports which came to hand, it was evident that in some cases it had become avirulent. Further inoculations with Tzaneen virus were accordingly discontinued, and I reverted back to the Ordinary strain. As this avirulency was contrary to previous experience, a thorough investigation was undertaken, but so far I am unable to adduce any reasons for it. Some 230 mules had been inoculated with this inert virus before any steps could be taken, and about 17 had died. The remainder were reinoculated with the Ordinary strain, and the chances of further mortality reduced to a minimum, also the owners of mules which had died owing to the inoculation with inert virus received compensation.

These inoculations and deaths from inert virus have not been included in any of the following tables, but all reinoculations have been taken into consideration.

(*a*) Results in the Transvaal.

Number of mules inoculated and number which died from July, 1906, to June, 1907.

District.	Number Inoculated.	Deaths.	Percentage.
Barberton	80	3	3·7
Krugersdorp	20	2	10·0
Lydenburg	280	6	2·1
Middelburg	133	4	3·0
Wakkerstroom	268	17	6·4
Potchefstroom	100	5	5·0
Pretoria District	804	35	4·3
Pretoria Experimental Station	236	13	5·5
Rustenburg	380	13	3·4
Waterberg	220	4	1·8
Marico	102	3	3·0
Zoutpansberg	536	20	3·7
Ermelo	198	12	6·0
Standerton	23	1	4·3
Heidelberg	11	0	0
Total	3,391	138	4·0

The following table gives the number of mules inoculated monthly in the various districts, excluding those immunised at this Station (236):—

Monthly inoculations.

Month.		Number of Mules.				
		On Hand.	Inoculated.	Discharged.	Which Died.	Remaining.
1906	July	25	76	26	10	65
	August	65	28	65	1	27
	September	27	43	28	1	41
	October	41	245	143	11	162
	November	162	367	253	15	261
	December	261	582	494	23	326
1907	January	326	705	513	21	497
	February	497	332	555	10	264
	March	264	423	413	12	262
	April	262	222	374	11	99
	May	99	54	144	7	2
	June	2	78	67	3	10
	Total	—	3,155	3,045	125	10

(*b*) Other Colonies.

Horse-sickness serum and virus was issued to several South African Colonies, and all the inoculations were performed by qualified officials.

Natal inoculated 1,170 mules, of which 59 died, or 5 per cent. (excluding deaths from inert virus).

Rhodesia immunised 972 mules, with deaths amounting to 21, or 2 per cent. (excluding casualties with inert virus).

35 mules were immunised in the Bechuanaland Protectorate, of which 3 died—8 per cent.

In the Orange River Colony 24 mules were inoculated, with the result that 1 died—4 per cent.

In Swaziland 76 mules were inoculated, of which 4 died—5.2 per cent.

The total being 2,277 inoculated, of which 88 died—3.8 per cent.

(*c*) Relapses amongst Immunised Mules when Exposed to Natural Infection.

Towards the end of the season, statistics were collected in regard to the number of mules which died after discharge.

The majority of deaths were reported, but the figures cannot be regarded as quite accurate, since in many cases the diagnosis of horse-sickness would appear to be doubtful.

For the purposes of comparison I have classified these deaths under the various districts, although in several instances mules have died in a different district to where they were immunised.

Statement showing number of mules inoculated in the Transvaal since November, 1905, together with the mortality during inoculation and after discharge.

District.	Number of Mules Inoculated from Nov., 1905, to June, 1907.	Number of Mules which died during Inoculation from Nov., 1905, to June, 1907.	Number of Immunised Mules which died after exposure from Nov., 1905, to June, 1907.	Total Number of Mules which died as a result of and after Inoculation.	Total per Cent. of Deaths to Number Inoculated.
					Per Cent.
Barberton	267	6	8	14	5
Krugersdorp ...	55	3	1	4	7
Lydenburg	386	10	12	22	6
Middelburg	204	6	5	11	5
Wakkerstroom ...	372	22	7	29	8
Potchefstroom ...	259	10	6	16	6
Pretoria	1,459	63	17	80	6
Rustenburg	873	31	16	47	5
Waterberg	391	14	4	18	5
Marico	150	4	0	4	3
Zoutpansberg ...	850	27	10	37	4
Heidelberg	17	—			0
Ermelo	198	12	3	15	8
Standerton	23	1	0	1	4
	5,498	209	89	298	5·4

(*d*) Spontaneous Cases of Horse-Sickness.

The 1906-1907 season was the worst experienced for many years, mules dying in districts which hitherto were considered as being almost free from the disease, and the mortality in the recognised horse-sickness districts being correspondingly high.

In Ermelo 800 horses were reported as dying from horse-sickness and 200 non-inoculated mules. In Potchefstroom (including Wolmaransstad and Bloemhof) 1,542 horses died, and 222 non-inoculated mules.

The following statement contains all the statistics received:—

Deaths amongst horses and non-inoculated mules from horse-sickness during 1906-7.

District.	Deaths Amongst		Total.
	Horses.	Non-Inoculated Mules.	
Barberton			81
Krugersdorp	58	12	70
Lydenburg	204	69	273
Potchefstroom	1,542	222	1,764
Pretoria	504	71	575
Rustenburg			295
Zeerust		—	300
Zoutpansberg	352	96	448
Swaziland		—	80
Heidelberg	537	64	601
Ermelo	800	200	1,000
Standerton			500
Lichtenburg	786		786
Middelburg (no returns)			
Waterberg ..			6,773
Piet Retief ..			
Wakkerstroom ..			

(*e*) Total Results.

The total number of mules inoculated in the Transvaal, Natal, Rhodesia, Orange River Colony, Bechuanaland Protectorate from November, 1905, to June, 1907, is 8,766, of which 329 died during inoculation—3.7 per cent.—and 112 died after exposure. That is to say, of 8,766 mules treated by our immunisation method, 8,325 survived the inoculation and were proof against natural infection, and in view of the severe nature of the disease last season, I consider these results very satisfactory.

"E."—FURTHER NOTES ON IMMUNITY IN HORSE-SICKNESS.

In my article, "The Immunity in Horse-Sickness," included in the Annual Report for last year, I stated that the mortality amongst immunised mules when exposed to natural infection amounted to 0.6 per cent., and expressed the opinion that the cause of this mortality was due to the presence of virus of varying virulency in different districts. In particular, one virus from Tzaneen and another from Bulawayo were referred to, each of which was of greater virulency than the virus used for previous immunisations, and known as Ordinary. As this question of immunity is of the utmost importance, I continued experimenting with various kinds of virus, and carefully noted the results obtained by testing animals with (*a*) the same, and (*b*) a different strain of virus to that with which they were immunised.

All the animals used for these experiments were treated in the same way: that is to say, an animal was injected with a strain of virus (for instance, Ordinary, or Tzaneen, etc.) of a particular generation. The result of this injection was taken from the temperature charts and recorded as either reaction or doubtful reaction, or reaction with dikkop, or reaction and died. All temperature reactions accompanied with piroplasma equi, or atypical horse-sickness reactions, suggesting the presence of a different disease, have been excluded. At a later date this animal was tested on its immunity against horse-sickness by the injection or infusion of virus of a certain generation. In the cases of testing by injection, 2 c.c. of virus was the minimum quantity used, and for infusion it varied from 350 c.c. to 45,000 c.c.

The results of these tests were again classified under reaction, doubtful reaction, etc.

Explanation of Tables.

In the following tables certain symbols, such as OTB and O-T-B appear, which must be explained. A virus or serum obtained by mixing the three strains, Ordinary, Tzaneen and Bulawayo, was, for the sake of brevity, designated O-T-B. But if the three vira were consecutively injected, and at the height of the fever reaction the animal was tapped and the blood utilised as virus, this trevalent virus was called OTB. Thus the symbol OTBLPW means that eight different vira—Ordinary, Tzaneen, Bulawayo, Lydenburg, Piet Retief, and Pietersburg (2) and Warmbaths—were consecutively injected, and an octovalent virus obtained from the animal during the fever reaction.

O, Ord., or Ordinary virus, represents a virus known as Ordinary, which was originally collected in the Pretoria District, and subsequently introduced into practice in November, 1905.

T, Tzn., or Tzaneen virus, represents a virus collected in Tzaneen, and which was introduced into practice in July, 1906.

B, Bul., or Bulawayo virus, represents a virus collected from a mule, which had died of horse-sickness at Bulawayo, but not yet introduced into practice.

O-T-B virus represents a mixture of the three vira Ordinary, Tzaneen and Bulawayo.

OTB virus represents a strain of virus obtained by consecutively injecting an animal with Ordinary, Tzaneen and Bulawayo virus, and at the height of horse-sickness reaction the animal was tapped and the blood used as virus (OTB).

OTBLPW virus represents a mixture obtained in the same way as OTB, but in addition contained virus collected from Lydenburg, Piet Retief, Pietersburg (2) and Warmbaths.

Dale virus represents a virus forwarded by Government Veterinary Surgeon Dale of Potchefstroom, and obtained from a mule which died of horse-sickness contracted spontaneously.

Edgar virus represents a virus forwarded by Government Veterinary Surgeon Edgar of Pietersburg, and obtained from a mule which died of horse-sickness contracted spontaneously.

Elder virus represents a virus forwarded by Government Veterinary Surgeon Elder of Swaziland, and obtained from a horse which died of horse-sickness contracted spontaneously.

Turnbull virus represents a virus forwarded by Government Veterinary Surgeon Turnbull, of Barberton, and obtained from a horse which died of horse-sickness contracted spontaneously.

Cape Colony virus represents a virus obtained from a mule which died of horse-sickness contracted spontaneously in the Cape Colony.

Altenroxel virus represents a virus obtained from various mules which died of horse-sickness, owner Mr. Altenroxel, of Tzaneen Estate, Pietersburg.

The full history of the treatment accorded to each animal is apparent from the tables, but a brief explanation may prove useful. An animal was immunised against horse-sickness on a certain date by the injection of 2 c.c. virus from a horse (origin either Ordinary, Tzaneen, etc.) of a particular generation and serum in varying quantities. The amount of serum is immaterial, and has been excluded in all the tables. The result of this injection of virus and serum was either (1) no reaction represented by —, (2) doubtful reaction represented by —, (3) reaction represented by R, (4) reaction accompanied with the lesions of dikkop, represented by RD; (5) reaction and died, represented R†; and (6) reaction accompanied with the lesions of dikkop and died, represented by RD†.

The animal was then subsequently tested, either by the injection of 2 c.c. to 5 c.c. virus, or by hyperimmunisation varying from 100 c.c. to 6 litres of a horse or mule (origin Ordinary, Tzaneen, etc., and of a certain generation).

The result was again classified under the headings Reaction, Doubtful Reaction, etc., and in cases where no result is given, naturally it means that the test failed to cause any temperature reaction, and no clinical symptoms were noted.

Second, third and subsequent tests are all given, the order in which they were made being shown by the date.

With the following exceptions the virus used for immunising or testing refers to virus obtained from horses:—

Virus 547 was obtained from a mule 547 (O strain).
Virus 548 ,, ,, ,, 548 ,,
Virus 561 ,, ,, ,, 561 ,,
Virus 659 ,, ,, ,, 659 ,,
Virus 666 ,, ,, ,, 666 ,,
Virus 701 ,, ,, ,, 701 ,,
Virus 1180 ,, ,, ,, 1180 ,,

Virus 1427 was obtained from a donkey previously injected with horse-sickness virus 726 (O strain).

Virus 1487 was obtained from a mule previously injected with virus which had passed through goats Nos. 375, 378 and 381.

Virus X was a mixture obtained from the blood of goats 375, 378 and 381, which had all previously been injected with horse virus 382.

Virus 1785 is a virus obtained from horse 1785 which had previously been injected with Turnbull virus (*q.v.*).

Virus 1788 is a virus obtained from mule 1788 which had previously been injected with Dale virus (*q.v.*)

Virus 1957 is a virus obtained from horse 1957 which had previously been injected with virus from mule 1772 (an animal inoculated with blood from a mule which died of horse-sickness contracted spontaneously in Warmbaths).

Virus 1418 and virus 2284.—Virus 2284 was obtained from mule 2284 which had been previously injected with virus from mule 1418 (an animal inoculated with blood from a case of horse-sickness in Cape Colony).

Virus 1506 is a virus obtained from horse 1506, which had previously been injected with blood of donkeys 1427, 1429, 1430 and 1433 (animals injected with O strain of virus).

Virus 1489 is a virus obtained from mule 1489, previously injected with blood from donkey 1599 (an animal injected with O strain of virus).

Virus 2169 and virus 2267 are two vira, the former obtained from horse 2169, and the latter from mule 2267, both animals having been inoculated with virus from donkey 1773 (an animal injected with Tzaneen virus—*q.v.*).

Virus 2201 is a virus obtained from horse 2201, an animal previously injected with blood from donkey 2208 (2208 was inoculated with Bulawayo virus—*q.v.*).

Immunisation of Mules with Ordinary Virus.

Mule No.	Immunisation. Date of Injection.	Virus. No.	Orig.	Gen.	Result.	Test. Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
	1902.					1903.		c.c.			
150	Nov. 26	216	Ord.	6	R	Feb. 9	262	250	Ord.	5	—
						March 5	278	500	,,	7	—
	1903.										
201	Jan. 10	227	,,	7	R	Feb. 20	265	100	,,	6	—
						April 8	278	250	,,	7	—
							295	500	,,	9	—
						1906.					
						Oct. 15	2199	5	Tzn.	12	—
						1903.					
320	June 4	300	,,	10	R	Aug. 17	354	500	Ord.	12	—
						Dec. 30	370	1000	,,	14	—
							310	1500	,,	10	—
341	July 23	335	,,	11		Aug. 17	354	500	,,	12	—
						Dec. 12	370	1000	,,	14	—
							390	1500	,,	17	—
368	Oct. 31	350	,,	15		Dec. 12	390	1500	,,	17	—
						Dec. 31	410	1000	,,	17	—
						1904.					
						May 5	512	1500	,,	24	—
						1903.					
371	Sept. 23	352	,,	14		Dec. 11	390	500	,,	17	—
	1904.					1904.					
412	Jan. 5	390	,,	17		Feb. 3	425	500	,,	20	—
						March 7	447	1000	,,	22	—
413	Feb. 15	419	,,	20	R	April 25	450	500	,,	22	—
						May 14	388	1500	,,	17	—
							387	2100	,,	25	—
415	Jan. 12	390	,,	17		Mar. 7	447	5	,,	22	—
						May 27	385	3000	,,	26	—
							520	300	,,	26	—
						Sept. 22	609	2000	,,	30	—
448	Mar. 1	446	,,	21	—	Nov. 1	557	8000	,,	33	—
						1905.					
						Oct. 14	1378	3000	,,	51	—
						Oct. 17	1385	6000	,,	50	—
						1904.					
459	,,	446	,,	21	—	Oct. 18	547	9750	,,	32	—
462	Feb. 29	446	,,	21	RD	April 25	481	1000	,,	23	—
							528	500	,,	25	—
						June 22	531	500	,,	25	—
							532	500	,,	26	—

R—Reaction. RD—Reaction with Dikkop.

Immunisation of Mules with Ordinary Virus.

Test.						Test.					
Date of Injection.	Virus.				Result.	Date of Injection.	Virus.				Result.
	No.	Qu.	Orig.	Gen.			No.	Qu.	Orig.	Gen.	
1903.		c.c.				1903.		c.c.			
April 8	295	1000	Ord.	9	–	Aug. 21	333	2000	Ord.	13	–
June 3	300	1500	,,	10	–	Oct. 9	350	2000	,,	15	
	316	2100	,,	11							
May 3	300	1000	,,	10		Aug. 11	338	1900	,,	12	
July 22	316	2000	,,	11		Oct. 9	352	2000	,,	14	
	335	1100	,,	11			350	2000	,,	15	
1901.						1906.					
Jan. 20	416	2000	,,	20		Oct. 15	2199	5	Tzn.	12	
May 5	116	2000	,,	21							
	512	2000	,,	24							
						1901.					
Jan. 20	414	1000	,,	19		May 5	512	2000	Ord.	21	
Feb. 24	419	2000	,,	20							
	116	1000	,,	21							
Feb. 3	119	1400	,,	20		April 9	423	1000	,,	22	–
March 20	417	1300	,,	22	–	April 26	181	1800	,,	23	–
	356	500	,,	17							
Oct. 1	561	2200	,,	30							
1901.											
Feb. 3	425	700	,,	20		April 27	481	1000	,,	23	–
April 9	117	1300	,,	22			478	1000	,,	23	
	423	1600	,,	22							
April 10	423	1000	,,	22	–	June 7	528	1500	,,	25	–
April 26	481	1000	,,	23			531	1500	,,	25	–
May 28	385	2500	,,	26		July 15	538	2300	,,	18	
June 29	520	2000	,,	26	–	Sept. 22	610	2000	,,	29	–
	406	3500	,,	17	–	Oct. 6	623	2000	,,	31	–
Mar. 30	167	1500	,,	22		April 25	456	1400	,,	24	–
						May 13	488	1300	,,	25	
Mar. 31	487	3000	,,	23			387	1500	,,	25	
Oct. 5	623	2200	,,	31	—						
Nov. 26	657	1600	,,	34							
1905.						1905.					
Feb. 24	705	5000	,,	40		June 26	1180	9000	,,	51	–
1906.											
Sept. 1	1965	5	Tzn.	1							
1905.											
Feb. 23	705	750	Ord.	40							
1904.						1904.					
June 21	529	500	,,	26		Aug. 5	581	2000	,,	27	–
	526	2000	,,	27			585	1000	,,	27	–
July 30	533	2000	,,	26		Sept. 9	534	1000	,,	27	–
							564	1000	,,	29	–

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

Mule No.	Immunisation. Date of Injection.	Virus. No.	Orig.	Gen.	Result.	Test. Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
						1905.		c.c.			
462 (*cont.*)						May 16	1064	9000	Ord.	46	
	1904.					1904.					
463	Feb. 29	446	Ord.	21	RD	April 25	478	1000	,,	23	—
							531	500	,,	25	—
						June 6	528	500	,,	25	—
						1905.					
						May 16	1067	9000	,,	46	
						1904.					
464	,,	446	,,	21	R	April 26	481	1000	,,	23	
							528	3000	,,	25	
						June 23	529	3500	,,	26	
465	,,	446	,,	21	RD	Oct. 10	626	1500	,,	31	—
466	,,	446	,,	21	R	Aug. 31	585	2000	,,	27	
							534	2000	,,	27	
468	,,	446	,,	21	RD	April 8	423	1500	,,	22	
							481	500	,,	23	
						June 5	478	500	,,	23	
							528	500	,,	25	
							531	500	,,	25	—
469	Mar. 19	447	,,	22		May 19	387	2000	,,	25	—
						May 30	385	2200	,,	26	—
479	April 5	447	,,	22	—	April 25	456	500	,,	24	—
							488	1700	,,	25	—
						May 14	387	2200	,,	25	
482	,,	447	,,	22	RD	April 27	478	1400	,,	23	—
							481	1500	,,	23	
						June 22	531	2500	,,	25	
							532	2500	,,	26	—
485	,,	447	,,	22	R	Oct. 21	548	10000	,,	32	—
						1905.					
						Oct. 14	1379	6000	,,	51	—
						Oct. 17	1386	3000	,,	50	—
						1904.					
486	April 5	447	,,	22	R	Sept. 7	536	3700	,,	28	—
492	April 25	423	,,	22	R	June 8	528	800	,,	25	—
							531	900	,,	25	
						Aug. 1	529	3000	,,	26	—
							533	1800	,,	26	—

R—Reaction. RD—Reaction with Dikkop.

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

Test.						Test.					
Date of Injection.	Virus.				Result.	Date of Injection.	Virus.				Result.
	No.	Qu.	Orig.	Gen.			No.	Qu.	Orig.	Gen.	
1905.		c.c.				1905.		c.c.			
Aug. 22	1270	1500	Ord.	45		Dec. 22	1532	3000	Ord.	60	
						Dec. 31	1531	2500	,,	60	
Aug. 23	1277	7500	,,	14			1611	3000	,,	40	
1901.						1904.					
June 21	532	500	,,	26		Aug. 8	582	2000	,,	27	
	529	500	,,	26			585	1000	,,	27	
July 28	526	2000	,,	27			580	1000	,,	29	
	533	1000	,,	26			561	2000	,,	30	
1905.						1906.					
Aug. 26	1272	9000	,,	15		Sept. 1	1965	5	Tzn.	1	
1904.											
July 30	533	2000	,,	26		Oct. 15	2199	5	,,	12	
Aug. 6	581	2500	,,	27							
Nov. 16	640	4500	,,	33		Nov. 23	657	2500	Ord.	31	
Sept. 11	580	2600	,,	29							
Sept. 30	561	2700	,,	30							
						1905.					
Aug. 30	531	2000	,,	27		May 5	1065	9000	,,	45	
	461	1000	,,	22		Aug. 22	1277	9000	,,	44	
	580	1000	,,	29							
	564	1000	,,	29							
						1904.					
June 29	406	2000	,,	17		Aug. 20	393	3000	,,	18	
July 16	538	2300	,,	18		Sept. 10	536	2500	,,	28	
May 28	385	2500	,,	26		July 16	538	4000	,,	18	—
	520	2000	,,	26		July 21	610	2200	,,	29	—
June 29	406	3000	,,	17							
						1906.					
						Oct. 15	2199	5	Tzn.	12	
July 7	536	2000	,,	28							
						1904.					
	533	2500	,,	26	—	Sept. 29	561	3000	Ord.	30	—
Aug. 7	581	3000	,,	27							
1905.						1905.					
Feb. 24	705	5000	,,	40		June 27	1194	8500	,,	38	
1906.											
Sept. 1	1965	5	Tzn.	1							
1904.											
Oct. 7	623	2000	Ord.	31							
						1904.					
Aug. 9	582	2000	,,	27	—	Sept. 16	564	2500	,,	29	—
Sept. 1	585	1800	,,	27	—	Sept. 23	609	2700	,,	30	—

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

Mule No.	Immunisation. Date of Injection.	Virus. No.	Orig.	Gen.	Result.	Test. Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
	1904					1904.		c.c.			
493	April 25	423	Ord.	22	R	Sept. 23	609	3300	Ord.	30	—
498	..	423	..	22	?	Sept. 8	536	1800	..	28	—
501	..	423	..	22	RD	May 30	385	2200	.	26	—
						to July	406	2000	..	17	—
							538	2500	..	18	—
509	..	423	..	22	RD	June 8	528	1700	..	18	—
						June 25	532	3000	..	26	—
530	Nov. 4	547	..	32	RD	Dec. 3	659	5950	..	35	—
						1905.					
						Oct. 18	1385	9000	..	50	—
						1904.					
417	Feb. 15	419	..	20	?	Aug. 18	586	2000	..	27	—
418	..	419	..	20	R	..	393	2500	..	18	—
429	Mar. 7	447	..	22	?	Aug. 20	393	3000	..	18	—
431	..	447	..	22	R	Aug. 31	585	3000	..	27	—
539	Nov. 12	547	..	32	R	Dec. 4	645	5350	..	35	—
552	Nov. 4	547	..	32	R	Dec. 3	645	4900	..	35	—
553	Nov. 16	640	..	33	?	Dec. 31	694	5000	..	36	—
554	Nov. 4	547	..	32	R	Dec. 3	659	7250	..	35	—
						1905.					
						Oct. 18	1386	9000	..	50	—
						1907.					
						Jan. 5	2411	9000	OTB LPW	1	—
						1904.					
555	Nov. 16	640	..	33	R	Dec. 30	694	1500	Ord.	36	—
559	Oct. 11	623	..	31	R	Dec. 3	659	6000	..	35	—
562	Oct. 10	623	..	31	?	Nov. 24	657	3500	..	34	—
565	Nov. 16	640	..	33	RD	Dec. 31	671	7500	..	37	—
566	..	640	..	33	R	..	666	6250	..	36	—
						1906.					
						Sept. 20	1965	5	Tzn.	1	—
						1905.					
567	Dec. 2	640	..	33	RD	Jan. 12	701	7500	Ord.	38	—
						Oct. 28	1393	2000	..	53	—
						Oct. 31	1395	7000	..	53	—
						1904.					
568	Nov. 16	640	..	33	R	Dec. 31	666	7500	..	36	—
						1905.					
						Oct. 17	1385	6000	..	50	—
						Oct. 19	1384	3000	..	52	—
						1904.					
570	Oct. 5	561	..	30	R	Dec. 3	557	3500	..	33	—
							645	5900	..	35	—
						1905.					
						Oct. 18	1386	8500	..	50	—

R –Reaction. ? – Doubtful. RD Reaction with Dikkop.

Immunisation of Mules with Ordinary Virus.—(*cont.*)

Test.						Test.					
Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.	Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.
1904.		c.c.						c.c.			
Oct. 10	623	2000	Ord.	30	—						
Oct. 9	626	2500	,,	31							
Sept. 10	536	2500	,,	28							
						1904.					
Aug. 4	533	1800	,,	26		Sept. 16	564	2200	Ord.	29	—
Aug. 9	581	2500	,,	27		Sept. 23	609	2700	,,	30	—
1905.						1905.					
Feb. 24	705	5000	,,	40		June 27	1184	8500	,,	51	—
1906.											
Oct. 17	2199	5	Tzn.	12	R						
1904.						1904.					
Sept. 23	609	3300	Ord.	30		Oct. 6	623	2300	,,	31	
Sept. 10	536	2500	,,	28							
Sept. 15	564	1000	,,	29		Oct. 23	547	2000	,,	32	—
1905.											
Feb. 12	705	7000	,,	10							
,,	705	5200	,,	40							
Jan. 28	714	2750	,,	38							
						1905.					
Feb. 24	706	5000	,,	10		June 26	1194	5500	,,	38	
						June 28	1180	3000	,,	51	—
1906.						1906.					
Sept. 20	1965	5	Tzn.	1	—	Oct. 15	2086	5	Bul.	5	
1905.											
Jan. 29	711	7495	Ord.	38	—						
Feb. 23	706	5000	,,	40							
1904.											
Dec. 31	694	5000	,,	36	—						
1905.											
Feb 25	706	5250	,,	40							
						1905.					
May 16	1064	8500	,,	46	—	Sept. 5	1279	9000	Ord.	46	—
May 30	1069	7000	,,	48	—	June 2	1117	2000	,,	48	—
Feb. 23	705	1000	,,	40	—	June 28	1184	8500	,,	51	—
1906.											
Sept. 20	1965	5	Tzn.	1							
1905.											
Feb. 24	701	3000	Ord.	38	—	June 26	1184	8000	,,	51	—
	706	3000	,,	40	—	June 28	1194	500	,,	38	—
1906.						1906.					
Sept. 1	1965	5	Tzn.	1	—	Oct. 15	2086	5	Bul.	5	—

R = Reaction.

Immunisation of Mules with Ordinary Virus.—(*cont.*)

Mule No.	Immunisation. Date of Injection.	Immunisation. Virus. No.	Orig.	Gen.	Result.	Test. Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
	1904.					1904.		c.c.			
571	Oct. 28	548	Ord.	32	?	Dec. 24	657	2500	Ord.	34	—
572	Nov. 5	547	,,	32	R	Dec. 12	703	7250		38	—
						1906.					
						Sept. 20	1965	5	Tzn.	1	—
						1904.					
573	Nov. 3	547	,,	32	R	Dec. 12	659	5750	Ord.	35	—
574	Nov. 16	640	,,	33	R	Dec. 31	671	7000	,,	37	—
						1906.					
						July 17	2058	10	Tzn.	6	RD
						1905.					
576	Dec. 2	640	,,	33	RD	Jan. 12	703	7500	Ord.	38	—
						Aug. 11	1869	1	Tzn.	1	—
							2060	1	Bul.	2	—
578	Oct. 20	547	,,	32	?	Jan. 12	703	7500	Ord.	38	—
						1906.					
						Sept. 20	1965	5	Tzn.	1	—
						1905.					
579	,,	547	,,	32	?	Jan. 12	701	7500	Ord.	38	—
						1906.					
						Sept. 20	1965	5	Tzn.	1	—
						1904.					
583	Nov. 16	640	,,	33	RD	Dec. 31	671	7500	Ord.	37	—
	1905.					1905.					
584	Jan. 8	694	,,	36	R	Feb. 8	747	6000	,,	39	—
	1904.										
589	Oct. 20	547	,,	32	R	Jan. 13	703	6800	,,	38	—
						1906.					
594	Dec. 30	694	,,	36	R	July 18	2058	10	Tzn.	6	R
						1905.					
629	Dec. 2	640	,,	36	RD	Mar. 21	892	9000	Ord.	41	—
632	,,	659	,,	35	R	,,	707	9500	,,	41	—
						1906.					
						Aug. 10	1869	1	Tzn.	1	—
							2060	1	Bul.	2	—
						1904.					
650	Nov. 16	640	,,	33	R	Dec. 31	666	7500	Ord.	36	—
651	,,	640	,,	33	R	Dec. 30	671	6000	,,	37	—
						1906.					
						Sept. 20	1965	20	Tzn.	1	—
						1904.					
654	,,	640	,,	33	R	Dec. 31	666	7750	Ord.	36	—
						1906.					
						Sept. 20	1965	5	Tzn.	1	—

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop.

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

Test.						Test.					
Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.	Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.
1904.		c.c.						c.c.			
Dec. 31	694	6500	Ord.	36	—						
1905.						1905.					
May 29	1070	5000	,,	18		Sept. 7	1279	9000	Ord.	46	—
June 22	1117	1000	,,	48	—						
1905.											
Feb. 11	705	4750	,,	40	—						
May 17	1064	9000	,,	46	—	Sept. 5	1278	3000	,,	46	—
						Sept. 8	1307	6000	,,	47	—
1906.											
Aug. 12	1996	9000	Tzu.	2	—						
1905.											
June 2	1070	3000	Ord.	48	—	Sept. 9	1281	9000	,,	47	—
	1117	5500	,,	48							
,,	1069	1500	,,	48	—	Sept. 8	1281	8000	,,	47	—
	1117	1500	,,	18	—	Sept. 13	1313	1000	,,	47	—
1906.											
Oct. 15	2086	5	Bul.	5	RD						
1905.											
June 5	1158	3000	Ord.	49	—	Sept. 27	1319	9000	,,	49	—
June 7	1118	3000	,,	48	—						
	1156	2500	,,	49	—						
1907.											
Mar. 15	Relapse of Dikkop										
1905.											
Feb. 23	706	3500	Ord.	40	—						
June 6	1156	9000	,,	49	—	,,	1319	9000	,,	49	—
1906.											
Aug. 19	2034	9000	Tzu.	3	—						
1905.											
June 27	1181	9000	Ord.	51	—	Oct. 18	1385	3000	,,	50	—
						Oct. 20	1387	6000	,,	52	
1906.											
Sept. 6	2196	9000	Bul.	5	—						
1905.											
May 9	1065	3000	Ord.	45	—						
May 16	1067	4500	,,	46	—						
May 9	1065	9000	,,	45	—	Aug. 23	1271	9000	,,	45	—
May 16	1067	8500	,,	46	—	Sept. 5	1278	7500	,,	46	—
						Sept. 6	1279	1500	,,	46	—

RD—Reaction with Dikkop.

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

Mule No.	Immunisation. Date of Injection.	Virus. No.	Orig.	Gen.	Result.	Test. Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
	1904.					1905.		c.c.			
660	Dec. 2	659	Ord.	35	RD	Jan. 25	670	7500	Ord.	37	—
662	,,	659	,,	35	R	,,	670	7500	,,	37	
663	,,	659	,,	35	RD	Jan. 24	670	7500	,,	37	—
664	,,	659	,,	35	R	,,	670	2750	,,	37	—
						Oct. 18	1386	9000	,,	50	—
665	,,	659	,,	35	RD	Jan. 11	701	4500	,,	38	—
672	Dec. 18	658	,,	35	R	Feb. 8	747	1500	,,	39	—
		658	,,	35	R	Jan. 28	711	7500	,,	38	—
681	,,					1906.					
	1905	701	,,	38	R	Oct. 15	2199	5	Tzn.	12	—
696	Jan. 12					1905.					
699	Jan. 8	666	,,	36	R	Feb. 9	747	7150	Ord.	39	—
						1906.					
						Aug. 10	2060	2	Bul.	2	RD
						1905.					
803	Jan. 26	726	,,	37	R	Mar. 20	707	6500	Ord.	41	—
						Mar. 21	892	3500	,,	41	—
						1906.					
						Aug. 10	2060	2	Bul.	2	RD
						1905.					
813	Feb. 28	726	,,	37	R	May 17	1064	2000	Ord.	46	—
						May 24	1068	8000	,,	47	—
814	,,	726	,,	37	R	July 3	1193	6500	,,	39	—
						July 4	1198	2500	,,	52	—
816	,,	726	,,	37	R	July 5	1193	8750	,,	39	—
817	,,	726	,,	37	R	May 23	1068	9000	,,	47	—
						1906.					
						Aug. 2	1964	10	Bul.	2	—
						1905.					
818	,,	726	,,	37	R	May 22	1068	4000	Ord.	47	—
821	,,	726	,,	37	RD	July 4	1198	8500	,,	52	—
822	,,	726	,,	37	RD	May 24	1072	9000	,,	47	—
826	,,	726	,,	37	R	May 22	1072	6000	,,	47	—
						May 29	1069	3000	,,	48	—
828	,,	726	,,	37	R	July 3	1198	5500	,,	52	—
						July 5	1197	3000	,,	52	—
829	,,	726	,,	37	R	June 5	1118	9000	,,	48	—

R—Reaction. RD—Reaction with Dikkop.

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

Test.						Test.					
Date of Injection.	Virus.				Result.	Date of Injection.	Virus.				Result.
	No.	Qu.	Orig.	Gen.			No.	Qu.	Orig.	Gen.	
1905.		c.c.				1905.		c.c.			
June 7	1156	7500	Ord.	49	—	Sept. 29	1368	9000	Ord.	49	—
June 5	1158	1500	,,	49	—						
June 8	1156	2000	,,	49	—						
	1157	5000	,,	49	—						
June 6	1158	8500	,,	49	—						
Mar. 21	892	7500	,,	41	—	June 26	1184	3000	,,	51	—
						July 5	1180	500	,,	51	—
							1179	5500	,,	52	—
1906.						1906.					
Sept. 20	1965	5	Tzn.	1	—	Dec. 30	2476	9000	OTB	1	—
1905.											
Feb. 12	705	7000	Ord.	40	—						
						1905.					
June 6	1156	9000	Ord.	49	—	Sept. 28	1368	9000	Ord.	49	—
1906.											
Aug. 30	2180	9000	Bul.	4	—						
1905.											
Mar. 27	1180	9000	Ord.	51	—	Oct. 19	1384	9000	,,	52	—
1906.											
Aug. 30	2180	9000	Bul.	4	—						
1905.						1906.					
Aug. 31	1282	6000	Ord.	45	—	Sept. 20	1965	5	Tzn.	1	—
Sept. 1	1275	3000	,,	45	—	Oct. 16	2086	5	Bul.	5	RD
Sept. 2	1282	9000	,,	45	—	July 18	2058	10	Tzn.	6	—
1906.											
Aug 22	2179	9000	Bul.	3	—						
1905.											
June 5	1118	5000	Ord.	48	—						
Oct. 20	1387	7000	,,	52	—						
Oct. 25	1391	2000	,,	52	—						
Sept. 15	1315	7500	,,	47	—	Sept. 20	1965	5	,,	1	
Sept. 18	1318	1500	,,	47	—						
Oct. 28	1394	9000	,,	53	—	Aug. 10	2060	2	Bul.	2	—
						,, 31	2180	9000	,,	4	—

RD—Reaction with Dikkop.

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

Mule No.	Immunisation. Date of Injection.	Immunisation. Virus. No.	Orig.	Gen.	Result.	Test. Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
831	1905. Feb. 28.	726	Ord.	37	RD	1905. July 4	1197	c.c. 7000	Ord.	52	—
						July 5	1193	2000	,,	39	—
833	,,	726	,,	37	R	May 22	1072	2000	,,	47	—
834	,,	726	,,	37	R	July 14	1183	9500	,,	53	—
835	,,	726	,,	37	R	July 4	1197	6000	,,	52	—
						July 5	1179	2000	,,	52	—
836	,,	726	,,	37	R	June 9	1157	8500	,,	49	—
837	,,	726	,,	37	R	July 6	1179	9000	,,	52	—
838	,,	726	,,	37	R	June 9	1157	1000	,,	49	—
						June 20	1185	8000	,,	50	—
839	,,	726	,,	37	R	,,	1185	9000	,,	50	—
841	,,	726	,,	37	R	July 4	1197	6000	,,	52	—
						July 6	1179	3000	,,	52	—
842	,,	726	,,	37	R	June 20	1189	9000	,,	50	—
843	,,	726	,,	37	R	,,	1189	8500	,,	50	—
844	,,	726	,,	37	R	,,	1190	9000	,,	50	—
845	,,	726	,,	37	R	July 4	1198	6500	,,	52	—
						July 6	1193	3000	,,	39	—
846	,,	726	,,	37	R	June 21	1190	9000	,,	50	—
847	,,	726	,,	37	R	July 14	1183	9000	,,	53	—
849	,,	726	,,	37	R	,,	1183	7500	,,	53	—
850	,,	726	,,	37	RD	July 17	1186	6000	,,	41	—
						July 21	1211	3000	,,	41	—
851	,,	726	,,	37	R	June 21	1188	9000	,,	50	—
852	,,	726	,,	37	R	July 17	1186	5000	,,	41	—
						July 21	1211	4000	,,	41	—
853	,,	726	,,	37	RD	June 20	1188	8500	,,	50	—
854	,,	726	,,	37	R	June 19	1188	500	,,	50	
						June 28	1189	1500	,,	50	
							1194	7000	,,	38	
856	Mar. 20	726	,,	37	R	July 21	1211	3000	,,	41	
						July 28	1199	3000	,,	52	
							1243	3000	,,	42	
857	,,	726	,,	37	R	July 24	1199	9000	,,	52	—
858	Feb. 28	726	,,	37	RD	June 20	1190	3000	,,	50	—
						July 5	1179	6000	,,	52	—

R—Reaction. RD—Reaction with Dikkop.

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

Test.						Test.					
Date of Injection.	Virus.				Result.	Date of Injection.	Virus.				Result.
	No.	Qu.	Orig.	Gen.			No.	Qu.	Orig.	Gen.	
1905.		c.c.				1906.		c.c.			
Oct. 28	1394	8500	Ord.	53		Sept. 1	1965	5	Tzn.	1	—
						Oct. 15	2086	5	Bul.	5	—
June 6	1158	6500	„	49	—						
Nov. 1	1396	9000	„	53		Aug. 10	2060	2	„	2	RD†
Oct. 26	1392	3000	„	52							
Oct. 27	1393	6000	„	53	-						
Sept. 28	1319	1000	„	49	—	Sept. 20	1965	5	Tzn.	1	—
Sept. 30	1371	8000	„	49	—						
Oct. 28	1393	3000	„	53	—						
Oct. 31	1395	6000	„	53	—						
Sept. 30	1369	9000	„	49	—	Oct. 15	2199	5	„	12	—
„	1369	8500	„	49	—	Sept. 1	1965	5	„	1	—
Oct. 28	1393	2000	„	53	—						
Nov. 1	1396	6500	„	53	—						
Oct. 2	1371	9000	„	49	—	Sept. 20	1965	5	„	1	—
Nov. 1	1395	9000	„	53	—						
„	1395	3000	„	53	—	Sept. 20	1965	5	„	1	—
Nov. 2	1396	6000	„	53	—	Oct. 15	2086	5	Bul.	5	RD
„	1396	9000	„	53	—	Sept. 1	1965	5	Tzn.	1	—
Nov. 7	1439	9000	„	54	—						
Oct. 20	1387	9000	„	52							
Nov. 15	1444	9000	„	56		Sept. 1	1965	5	„	1	R
Nov. 1	1395	3000	„	53	—	Oct. 17	2199	5	„	12	R
Nov. 6	1437	6000	„	54	—	Nov. 11.	1964	2	Bul.	2	—
Oct. 21	1384	9000	„	52	-	Sept. 20	1965	5	Tzn.	1	Tzn.
						Oct. 15	2086	5	Bul.	5	RD†

R—Reaction. RD†—Reaction with Dikkop and died.

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

Mule No.	Immunisation. Date of Injection.	Virus. No.	Orig.	Gen.	Result.	Test. Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
	1905.					1905.		c.c.			
860	Feb. 28.	726	Ord.	37	R	June 20	1185	1500	Ord.	50	—
						June 27	1181	5000	,,	51	
						June 28	1180	2500	,,	51	
861	,,	726	,,	37	R	July 17	1186	5000	,,	41	
						July 21	1211	3500	,,	41	
862	,,	726	,,	37	R	June 20	1189	6500	,,	50	
						June 28	1180	2500	,,	51	
883	Mar. 20	726	,,	37	R	July 25	1199	9000	,,	52	
884	,,	726	,,	37	R	July 24	1199	2500	,,	52	
						July 28	1243	6500	,,	42	—
885	,,	726	,,	37	RD	July 29	1243	9000	,,	42	
886	,,	726	,,	37	R	May 2	993	9000	,,	45	
						1906.					
						July 18	2058	10	Tzu.	6	
						1905.					
887	,,	726	,,	37	RD	July 29	1243	3000	Ord.	42	
						July 31	1187	6000	,,	42	
888	,,	727	,,	5	R	July 29	1243	3000	,,	42	
						Aug. 1	1196	6000	,,	42	
889	,,	727	,,	5	R	,,	1187	9000	,,	42	
891	,,	726	,,	37	R	Aug. 1	1196	6000	,,	42	—
						Aug. 9	1192	3000	,,	43	—
893	,,	726	,,	37	RD	Aug. 1	1196	9000	,,	42	
894	,,	726	,,	37	RD	,,	1187	6000	,,	42	—
						Aug. 8	1203	3000	,,	43	
895	,,	726	,,	37	R	Aug. 1	1196	6000	,,	42	
						Aug. 9	1200	3000	,,	43	
896	,,	726	,,	37	R	,,	1192	9000	,,	43	
897	,,	726	,,	37	R	,,	1203	9000	,,	43	
898	,,	726	,,	37	R	Aug. 10	1200	9000	,,	43	—
901	,,	726	,,	37	R	Aug. 9	1192	9000	,,	43	
903	,,	726	,,	37	R	Aug. 10	1203	9000	,,	43	
904	,,	726	,,	37	R	,,	1203	9000	,,	43	
905	,,	726	,,	37	RD	Aug. 14	1201	3000	,,	44	
						Aug. 15	1266	6000	,,	44	
906	,,	726	,,	37	R	Aug. 12	1201	6000	,,	44	
						Aug. 15	1266	3000	,,	44	—
907	,,	726	,,	37	R	Oct. 7	1373	9000	,,	50	
908	,,	726	,,	37	R	Oct. 25	1390	9000	,,	52	—
909	,,	727	,,	37	R	Sept. 17	1315	9000	,,	47	

R—Reaction. RD—Reaction with Dikkop.

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

Test.						Test.					
	Virus.						Virus.				
Date of Injection.	No.	Qu.	Orig.	Gen.	Result.	Date of Injection.	No.	Qu.	Orig.	Gen.	Result.
1905.		c.c.						c.c.			
Oct. 20	1384	6000	Ord.	52							
Oct. 24	1390	3000	,,	52	—						
						1906.					
Nov. 7	1439	9000	,,	54		Sept. 20	1965	5	Tzn.	1	—
Oct. 21	1384	3000	,,	52	—	,,	1965	5	,,	1	—
Oct. 24	1391	6000	,,	52	—						
Nov. 7	1437	9000	,,	54	—						
Nov. 10	1440	4500	,,	55	—						
Nov. 14	1443	4500	,,	56	—						
Nov. 15	1443	9000	,,	56	—	Aug. 10	1869	1	,,	1	R
							2060	1	Bul.	2	R
						1905.					
Aug. 10	1192	3000	,,	43	—	Nov. 17	1444	6000	Ord.	56	—
Aug. 12	1201	6000	,,	44	—	Nov. 28	1490	3000	,,	57	
1906.											
Aug. 12	1996	9000	Tzn.	2	—						
1905.						1906.					
Nov. 7	1437	6000	Ord.	54	—	Sept. 20	1965	5	Tzn.	1	—
Nov. 8	1440	3000	,,	55	—						
Nov. 7	1437	3000	,,	54	—	,,	1965	5	,,	1	—
Nov. 10	1440	6000	,,	55	—						
Nov. 15	1444	9000	,,	55		,,	1965	5	,,	1	—
						Oct. 15	2086	5	Bul.	5	RD
Nov. 15	1443	6000	,,	56		Sept. 20	1965	5	Tzn.	1	—
Nov. 16	1444	3000	,,	56	—						
,,	1443	9000	,,	56	—	Oct. 15	2199	5	,,	12	—
Nov. 7	1439	9000	,,	54	—	Sept. 20	1965	5	,,	1	—
Nov. 16	1444	9000	,,	56	—						
Nov. 19	1444	9000	,,	56	—						
Nov. 17	1443	3000	,,	56	—						
Nov. 29	1490	6000	,,	57	—						
Dec. 6	1491	9000	,,	58	—	,,	1965	5	,,	1	—
Nov. 17	1443	3000	,,	56	—	,,	1965	5	,,	1	—
Dec. 5	1490	3000	,,	57	—						
	1491	3000	,,	58	—						
Dec. 12	1530	9000	,,	59	—	,,	1965	5	,,	1	
Dec. 13	1530	9000	,,	59		Sept. 1	1965	5	,,	1	RD
Dec. 12	1530	9000	,,	59		Sept. 20	1965	5	,,	1	—
1906.											
Mar. 19	1677	3000	,,	61	—	Sept. 1	1965	5	,,	1	—
Mar. 24	1883	6000	Dale	2	—						
Mar. 15	1779	9000	Ord.	61	—	Sept. 20	1965	5	,,	1	

R = Reaction. RD—Reaction with Dikkop.

Immunisation of Mules with Ordinary Virus.—(*cont.*)

Mule No.	Immunisation. Date of Injection.	Immunisation. Virus. No.	Orig.	Gen.	Result.	Test. Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
					R	1905		c.c.			
911	1905. Mar. 20	726	Ord.	37		April 18	1051	9000	Ord.	43	—
						1906. Sept. 20	1965	5	Tzn.	1	—
					R						
912	,,	726	,,	37		,,	1965	5	,,	1	—
					R	1905.					
913	,,	726	,,	37		Aug. 14	1201	3000	Ord.	44	—
					R	Aug. 16	1266	6000	,,	44	—
914	,,	726	,,	37		Sept. 15	1315	6000	,,	47	—
					R	Sept. 18	1318	3000	,,	47	
915	,,	726	,,	37		Oct. 9	1374	9000	,,	50	—
					R	1906.					
916	,,	726	,,	37		July 18	2058	10	Tzn.	6	R
					RD	1905.					
917	,,	726	,,	37	R	Sept. 18	1318	9000	Ord.	47	—
918	,,	726	,,	37	R	April 18	1052	9000	,,	43	—
919	,,	727	,,	5	R	Sept. 19	1318	9000	,,	47	—
920	,,	727	,,	5	R	April 18	1052	9000	,,	43	—
922	,,	726	,,	37		Sept. 15	1314	3000	,,	47	—
					R	Sept. 19	1316	6000	,,	48	—
923	,,	726	,,	37		Oct. 7	1373	9000	,,	50	—
					R						
924	,,	726	,,	37		Sept. 15	1314	2000	,,	47	—
						Sept. 20	1317	7000	,,	48	—
					RD	1906.					
925	,,	726	,,	37		Oct. 15	2199	5	Tzn.	12	—
					RD	1905.					
926	,,	726	,,	37	R	Sept. 21	1316	9000	Ord.	48	—
927	,,	726	,,	37	R	Sept. 20	1316	8500	,,	48	—
928	,,	726	,,	37		Aug. 19	1269	9000	,,	43	—
					R						
929	,,	727	,,	5		May 2	970	9000	,,	45	—
					R						
930	,,	727	,,	5		Sept. 20	1317	9000	,,	48	—
931	,,	726	,,	37	R	April 25	1048	6000	,,	44	—
						April 30	970	3500	,,	45	—
						1906. Oct. 15	2199	5	Tzn.	12	—
						1905.					
932	,,	726	,,	37	R	May 2	970	7000	Ord.	45	—
933	,,	726	,,	37	R	Sept. 21	1317	9000	,,	48	—
935	,,	726	,,	37	R	Aug. 18	1268	6000	,,	43	—
						Aug. 19	1269	3000	,,	43	—
936	,,	726	,,	37	RD	,,	1269	8500	,,	13	—
937	,,	726	,,	37	RD	Sept. 21	1316	9000	,,	48	—
938	,,	726	,,	37	R	Oct. 24	1391	9000	,,	52	—
939	,,	727	,,	5	RD	Aug. 22	1270	9000	,,	45	—

R—Reaction. RD—Reaction with Dikkop.

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

TEST.						TEST.					
Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.	Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.
1905. Aug. 10	1200	c.c. 9000	Ord.	43	—	1906. Jan. 2	1611	c.c. 9000	Ord.	40	—
1906. Oct. 15	2086	5	Bul.	5	R						
1905. Dec. 20	1546	7000	Ord.	60	—	Sept. 20	1965	5	Tzn.	1	—
1906. Mar. 14	1779	9000	,,	61	—	,,	1965	5	,,	1	—
Mar. 16	1779	9000	,,	61	—	Oct. 15	2199	5	,,	12	—
,,	1786	3000	,,	61	—	Sept. 20	1965	5	,,	1	—
Mar. 18	1677	6000	,,	61							
Mar. 19	1677	3000	,,	61	—	Oct. 15	2199	5	,,	12	—
Mar. 24	1883	6000	Dale	2							
Mar. 17	1677	9000	Ord.	61	—	,,	2199	5	,,	12	—
Mar. 16	1786	9000	,,	61	—	,,	2199	5	,,	12	—
Mar. 17	1677	9000	,,	61	—	,,	2199	5	,,	12	—
1905. Aug. 19	1268	9000	,,	43	—						
1906. Mar. 25	1883	5500	Dale	2	—	,,	2199	5	,,	12	—
Mar. 26	1885	3500	,,	2	—						
1905. Aug. 19	1268	9000	Ord.	43	—	Feb. 1	1647	9000	Ord.	38	—
1907. Jan. 5	2411	9000	OTB LPW	1	—						
1905. Aug. 19	1269	9000	Ord.	43	—						
1906. Mar. 25	1883	6000	Dale	2	—						
Mar. 26	1885	3000	,,	2	—						
Jan. 17	1489	9000	Ord.	61	—	Oct. 15	2199	5	Tzn.	12	—
,,	1489	9000	,,	61	—	,,	2199	5	,,	12	—
Mar. 27	1883	4500	Dale	2	—	Sept. 1	1965	5	,,	1	—
April 13	1896	3500	Edgar	1	—						
1905. Dec. 22	1531	9000	Ord.	60	—						

R—Reaction.

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

Mule No.	Immunisation.					Test.					
	Date of Injection.	Virus.			Result.	Date of Injection.	Virus.				Result.
		No.	Orig.	Gen.			No.	Qu.	Orig.	Gen.	
	1905.					1905.		c.c.			
940	Mar. 20	727	Ord.	5	RD	Sept. 23	1365	9000	Ord.	48	—
941	,,	726	,,	37	R	,,	1366	9000	,,	48	—
						1906.					
942	April 11	726	,,	37	R	July 18	2058	10	Tzn.	6	R
						1905.					
945	,,	726	,,	37	R	Aug. 23	1271	2500	Ord.	45	—
						Aug. 25	1269	6000	,,	43	—
946	,,	726	,,	37	R	Oct. 25	1391	6000	,,	52	—
						Oct. 26	1392	3000	,,	52	—
947	,,	726	,,	37	R	Aug. 23	1270	6000	,,	45	—
						Aug. 25	1267	3000	,,	45	
948	,,	726	,,	37	R	Oct. 25	1390	9000	,,	52	
949	,,	726	,,	37	R	Nov. 30	1490	9000	,,	57	—
950	,,	726	,,	37	R	Aug. 22	1270	9000	,,	45	—
952	,,	726	,,	37	R	,,	1271	9000	,,	45	—
955	,,	726	,,	37	R	Oct. 9	1374	9000	,,	50	—
956	,,	726	,,	37	R	Sept. 23	1365	9000	,,	48	—
957	,,	726	,,	37	RD	May 16	1065	3	,,	45	—
						1906.					
						Feb. 1	1647	2500	,,	38	—
						Feb. 6	1770	6000	,,	63	—
						1905.					
958	,,	726	,,	37	R	April 23	1277	9000	,,	44	—
959	,,	726	,,	37	R	Aug. 22	1271	9000	,,	45	—
960	,,	726	,,	37	R	Oct. 9	1373	7000	,,	50	—
						Oct. 11	1375	2000	,,	51	—
962	,,	726	,,	37	R	Aug. 30	1273	9000	,,	45	—
963	,,	726	,,	37	RD	,,	1273	9000	,,	45	—
964	,,	726	,,	37	R	Sept. 23	1366	9000	,,	48	—
965	,,	726	,,	37	R	May 16	1065	3	,,	45	—
						Sept. 7	1769	7500	,,	63	—
						Sept. 13	1771	1500	,,	62	—
967	,,	726	,,	37	R	Aug. 31	1274	9000	,,	45	
968	,,	726	,,	37	R	Aug. 25	1267	6000	,,	45	—
						Aug. 26	1272	3000	,,	45	—
969	,,	726	,,	37	R	Sept. 1	1275	9000	,,	45	—
971	,,	726	,,	37	RD	Sept. 30	1371	9000	,,	49	—
972	,,	726	,,	37	R	Sept. 23	1365	8500	,,	48	—
973	,,	726	,,	37	RD	May 16	1065	3	,,	45	—
						1906.					
						Feb. 14	1771	9000	,,	62	—
						1905.					
974	,,	726	,,	37	R	Sept. 23	1366	3000	,,	48	—
						Sept. 26	1365	6000	,,	48	—

R—Reaction. RD—Reaction with Dikkop.

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

TEST.						TEST.					
Date of Injection.	Virus.				Result.	Date of Injection.	Virus.				Result.
	No.	Qu.	Orig.	Gen.			No.	Qu.	Orig.	Gen.	
1906.		c.c.				1906.		c.c.			
Jan. 18	1489	9000	Ord.	61	—	Oct. 15	2199	5	Tzn.	12	—
Jan. 18	1489	6000	,,	61	—	,,	2199	5	,,	12	—
Feb. 1	1709	1500	,,	39							
Aug. 2	1964	10	Bul.	2	R	Aug. 24	2179	9000	Bul.	3	
Feb. 1	1647	8000	Ord.	38	—	Oct. 15	2199	5	Tzn.	12	—
Sept. 1	1965	5	Tzn.	1	—						
1905.											
Nov. 30	1490	9000	Ord.	57		,,	2199	5	,,	12	R†
1906.											
April 17	1896	3000	Edgar	1		Sept. 1	1965	5	,,	1	—
July 18	2058	10	Tzn.	6	R	Aug. 15	1995	9000	,,	2	—
Jan. 15	1489	3000	Ord.	61	—						
,,	1489	9000	,,	61		Oct. 15	2199	5	,,	12	—
Mar. 27	1883	2500	Dale	2	—	,,	2199	5	,,	12	—
Mar. 28	1885	6000	,,	2	—						
1905.						1905.					
June 23	726	5	Ord.	37	—	Aug. 31	1273	3000	Ord.	45	—
1906.							1276	6000	,,	45	—
Oct. 15	2199	5	Tzn.	12							
1905.						1906.					
Dec. 22	1532	6000	Ord.	60	—	Oct. 15	2199	5	Tzn.	12	—
Dec. 31	1611	3000	,,	40							
1906.											
Mar. 16	1786	9000	,,	61	—	,,	2199	5	,,	12	—
Feb. 5	1769	9000	,,	63	—	,,	2199	5	,,	12	—
Feb. 6	1770	9000	,,	63	—	,,	2199	5	,,	12	—
Mar. 28	1885	8000	Dale	2		,,	2199	5	,,	12	—
1905.						1905.					
June 23	726	5	Ord.	37	—	Aug. 31	1274	9000	Ord.	45	—
1906.											
Oct. 15	2199	5	Tzn.	12	—						
						1906.					
Feb. 7	1770	9000	Ord.	63	—	Oct. 15	2199	5	Tzn.	12	—
Feb. 1	1709	9000	,,	39	—	,,	2199	5	,,	12	—
Feb. 7	1770	9000	,,	63	—	,,	2199	5	,,	12	—
April 14	1896	9000	Edgar	1	—	,,	2199	5	,,	12	—
1905.						1905.					
June 23	726	5	Ord.	37	—	Sept. 2	1282	3000	Ord.	45	—
1906.						Sept. 5	1278	6000	,,	46	—
Oct. 15	2199	5	Tzn.	12							
,,	2199	5	,,	12	—						

R—Reaction. R†—Reaction and Died.

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

Mule No.	Immunisation. Date of Injection.	Virus. No.	Orig.	Gen.	Result.	Test. Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
	1905.					1905.		c.c.			
975	April 11	726	Ord.	37	R	Sept. 1	1275	9000	Ord.	45	—
976	,,	726	,,	37	R	Dec. 6	1492	9000	,,	58	—
977	,,	726	,,	37	R	Nov. 30	1490	9000	,,	57	—
979	,,	726	,,	37	RD	Sept. 26	1365	9000	,,	48	—
980	,,	726	,,	37	RD	Oct. 9	1375	9000	,,	51	—
981	,,	726	,,	37	R	Aug. 26	1272	9000	,,	45	—
982	,,	726	,,	37	R	Sept. 1	1276	9000	,,	45	—
983	,,	726	,,	37	R	Sept. 26	1365	9000	,,	48	—
984	,,	726	,,	37	R	Aug. 30	1272	6000	,,	45	—
							1273	3000	,,	45	
985	,,	726	,,	37	R	Sept. 30	1368	9000	,,	49	—
987	,,	726	,,	37	R	Aug. 26	1267	9000	,,	45	—
						1906.					
						Dec. 30	2477	9000	OTB LPW	1	—
						1905.					
988	,,	726	,,	37	RD	Aug. 31	1274	3000	Ord.	45	—
						Sept. 1	1282	5500	,,	45	
989	,,	726	,,	37	R	Aug. 31	1273	3000	,,	45	—
						Sept. 5	1279	6000	,,	46	—
990	,,	726	,,	37	R	Oct. 12	1375	9000	,,	51	—
						1906.					
						Aug. 15	1995	9000	Tzn.	2	—
						1905.					
991	,,	726	,,	37	RD	Dec. 6	1492	9000	Ord.	58	—
						1906.					
						Nov. 11	1964	2	Bul.	2	—
						1905.					
992	,,	726	,,	37	R	Oct. 25	1391	4500	Ord.	52	—
						Oct. 26	1393	4000	,,	53	—
994	,,	726	,,	37	R	Sept. 28	1319	3000	,,	49	—
995	,,	726	,,	37	R	Sept. 2	1276	3000	,,	45	—
						Sept. 5	1280	6000	,,	46	—
						1906.					
						July 18	2058	10	Tzn.	6	R
						1905.					
996	,,	726	,,	37	R	Sept. 1	1276	9000	Ord.	45	—
997	,,	726	,,	37	R	Sept. 5	1276	3000	,,	45	—
							1280	6000	,,	46	—
						1906.					
						Aug. 18	2034	9000	Tzn.	3	—
						1905.					
999	,,	726	,,	37	R	Sept. 2	1275	6000	Ord.	45	—
						Sept. 5	1278	3000	,,	46	—
						1906.					
1000	,,	726	,,	37	R	Jan. 2	1611	9000	,,	40	—
						1905.					
1003	,,	726	,,	37	R	Oct. 27	1393	9000	,,	53	—
1004	,,	726	,,	37	R	Sept. 9	1307	9000	,,	47	—
						1906.					
						Sept. 20	1965	5	Tzn.	1	—

R—Reaction. RD—Reaction with Dikkop.

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

Test.						Test.					
Date of Injection.	Virus.				Result.	Date of Injection.	Virus.				Result.
	No.	Qu.	Orig.	Gen.			No.	Qu.	Orig.	Gen.	
1906.		c.c.						c.c.			
Mar. 6	1778	2000	Ord.	60	—						
						1906.					
July 18	2058	10	Tzn.	6	R	Aug. 13	1996	9000	Tzn.	2	—
Oct. 25	2199	5	,,	12	—	Nov. 11	1959	2	Bul.	3	—
April 15	1896	8500	Edgar	1	—	Aug. 10	1869	2	Tzn.	1	—
Feb. 1	1709	6000	Ord.	39	—	Aug. 10	2060	2	Bul.	2	—
Feb. 5	1769	3000	,,	63	—	Oct. 15	2199	5	Tzn.	12	—
Feb. 14	1771	9000	,,	62	—	,,	2199	5	,,	12	—
April 15	1897	9000	Dale	3	—	,,	2199	5	,,	12	—
1905.											
Dec. 23	1532	6000	Ord.	60	—	,,	2199	5	,,	12	—
,,	1351	3000	,,	38	—						
1906.											
Feb. 14	1771	9000	,,	62	—	,,	2199	5	,,	12	—
Feb. 15	1771	6000	,,	62	—	,,	2199	5	,,	12	—
Feb. 17	1650	3000	,,	62	—						
April 15	1897	9000	Dale	3	—	July 18	2058	10	,,	6	—
1905.											
Dec. 22	1532	3000	Ord.	60	—	Oct. 15	2199	5	,,	12	—
1906.											
Aug. 10	2060	2	Bul.	2	—	Sept. 6	2196	9000	Bul.	5	—
Feb. 15	1771	3000	Ord.	62	—	Feb. 27	1789	3000	Ord.	64	—
Feb. 17	1650	3000	,,	62	—						
Aug. 2	1964	10	Bul.	2	—	Aug. 23	2179	9000	Bul.	3	—
Feb. 28	1789	9000	Ord.	64	—	Oct. 15	2199	5	Tzn.	12	—
,,	1789	9000	,,	64	—	July 18	2058	10	,,	6	—
,,	1789	5500	,,	64	—	Oct. 15	2199	5	,,	12	—
Mar. 7	1778	3000	,,	60	—						
Mar. 18	1677	6000	,,	61	—	,,	2199	5	,,	12	—
Mar. 24	1883	3000	Dale	2	—						
Sept. 20	1965	5	Tzn.	1	—						
Mar. 27	1885	6000	Dale	2	—	April 11	1895	3000	Dale	3	—
Oct. 15	2086	5	Bul.	5	R	Nov. 11	1964	2	Bul.	2	—

R—Reaction.

Immunisation of Mules with Ordinary Virus.—(*cont.*)

Mule No.	Immunisation. Date of Injection.	Immunisation. Virus. No.	Orig.	Gen.	Result.	Test. Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
								c.c.			
	1905.					1905.					
1008	April 11	726	Ord.	37	RD	Oct. 13	1376	9000	Ord.	51	—
1010	,,	726	,,	37	RD	Sept. 8	1281	3000	,,	47	
						Sept. 10	1307	3000	,,	47	
						,, 12	1313	3000	,,	47	
1012	,,	726	,,	37	RD	Oct. 2	1371	4500	,,	49	
						Oct. 5	1370	4500	,,	49	
1013	,,	726	,,	37	R	Oct. 25	1392	6000	,,	52	—
						Oct. 27	1393	3000	,,	53	
1014	,,	726	,,	37	R	Oct. 12	1377	8500	,,	51	
1015	,,	726	,,	37	RD	Oct. 2	1369	3000	,,	49	
						Oct. 6	1370	5500	,,	49	
1016	,,	726	,,	37	R	Dec. 7	1491	9000	,,	58	
1018	,,	726	,,	37	R	Sept. 12	1312	9000	,,	47	—
1020	,,	726	,,	37	RD	Sept. 13	1312	9000	,,	47	
1021	,,	726	,,	37	R	,,	1313	9000	,,	47	
1022	,,	726	,,	37	R	Sept. 12	1314	9000	,,	47	
1023	,,	726	,,	37	R	Dec. 7	1492	8500	,,	58	
1024	,,	726	,,	37	R	Sept. 13	1313	9000	,,	47	
1025	,,	726	,,	37	R	Oct. 26	1392	3000	,,	52	—
						Oct. 27	1394	6000	,,	53	
1026	,,	726	,,	37	R	Sept. 13	1312	9000	,,	47	
1027	,,	726	,,	37	R	Oct. 13	1376	9000	,,	51	—
						1906.					
1028	,,	726	,,	37	RD	June 23	1964	8000	Bul.	2	RD†
						1905.					
1029	,,	726	,,	37	R	Oct. 4	1370	2500	Ord.	49	
						Oct. 6	1372	5000	,,	49	
1030	,,	726	,,	37	R	Oct. 13	1377	9000	,,	51	—
						1906.					
1031	,,	726	,,	37	R	June 23	1964	8500	Bul.	2	RD†
						1905.					
1034	,,	726	,,	37	R	Oct. 6	1372	6000	Ord.	49	
1035	,,	726	,,	37	R	,,	1370	1000	,,	49	
1036	,,	726	,,	37	R	Oct. 13	1375	9000	,,	51	—
1037	,,	726	,,	37	R	,,	1375	9000	,,	51	—
1038	,,	726	,,	37	R	Oct. 12	1377	3500	,,	51	—
						Oct. 14	1378	5500	,,	51	—
1039	,,	726	,,	37	R	,,	1376	3500	,,	51	—
						Oct. 15	1378	3000	,,	51	—
							1379	3000	,,	51	—
1040	,,	726	,,	37	R	Sept 12	1314	9000	,,	47	—
1041	,,	726	,,	37	R	Oct. 14	1378	9000	,,	51	—
1042	,,	726	,,	37	R	,,	1379	9000	,,	51	—
1043	,,	726	,,	37	R	,,	1379	8500	,,	51	—
1044	,,	726	,,	37	RD	Sept. 13	1314	6000	,,	47	—
							1313	3000	,,	47	—
						1906.					
						Sept. 5	2196	1000	Bul.	5	—

R—Reaction. RD—Reaction with Dikkop. RD†—Reaction with Dikkop and died.

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

Test.						Test.					
Date of Injection.	Virus.				Result.	Date of Injection.	Virus.				Result.
	No.	Qu.	Orig.	Gen.			No.	Qu.	Orig.	Gen.	
		c.c.						c.c.			
1906.											
Sept. 1	1965	5	Tzn.	1							
						1906.					
Mar. 27	1885	3000	Dale	2		April 13	1896	6000	Edgar	1	—
						Oct. 15	2199	5	Tzn.	12	—
Sept. 1	1965	5	Tzn.	1							
Sept. 20	1965	5	,,	1		Nov. 11	1964	2	Bul.	2	—
Oct. 15	2199	5	,,	12							
Sept. 20	1965	5	,,	1		Nov. 11	1959	2	,,	3	—
Aug. 10	2060	2	Bul.	2	R	Sept. 6	2196	8500	Tzn.	5	
April 12	1895	6000	Dale	3	—	Sept. 20	1965	5	,,	1	—
April 13	1896	3000	Edgar	1	—						
April 17	1896	3000	,,	1	—	Sept. 1	1965	5	,,	1	—
Sept. 20	1965	5	Tzn.	1	—	Nov. 11	1959	2	Bul.	3	
Oct. 15	2199	5	,,	12							
,,	2199	5	,,	12							
Aug. 10	1869	1	,,	1							
	2060	1	Bul	2	—						
Oct. 15	2199	5	Tzn.	12	—						
Sept. 1	1965	5	,,	1							
1905.											
Oct. 11	1376	1500	Ord.	51		Aug. 10	1869	1	Tzn.	1	R
							2060	1	Bul.	2	R
Oct. 11	1376	3000	,,	51	—						
Oct. 13	1376	8000	,,	51	—	Sept. 1	1965	5	Tzn.	1	RD
1906.											
Oct. 15	2199	5	Tzn.	12	—	Nov. 11	1959	2	Bul.	3	—
Sept. 1	1965	5	,,	1	—						
,,	1965	5	,,	1	—						
Sept. 20	1965	5	,,	1	—	,,	1964	5	,,	2	RD†
Sept. 1	1965	5	,,	1	R	Oct. 15	2086	5	,,	5	R
July 18	2058	10	,,	6	R	Aug. 2	1964	10	,,	2	?
Sept. 13	2266	8000	Bul.	3	—						

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop. RD†—Reaction with Dikkop and died.

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

Mule No.	Immunisation. Date of Injection.	Virus. No.	Orig.	Gen.	Result.	Test. Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
	1905.					1905.		c.c.			
1045	April 11	726	Ord.	37	R	Dec. 7	1491	12000	Ord.	58	- -
						1906.					
1179	June 30	1180	,,	51	R	Oct. 15	2199	5	Tzn.	12	- -
1195	,,	1180	,,	51	R	,,	2199	5	,,	12	
						1905.					
1212	July 14	726	,,	37	R	Sept. 6	1280	9000	Ord.	46	
1213	,,	726	,,	37	R	,,	1280	3000	,,	46	-
						Sept. 11	1281	3000	,,	47	
							1312	3000	,,	47	
1407	Oct. 20	726	,,	37	RD	Dec. 17	1492	6000	,,	58	-
						Dec. 18	1546	3000	,,	60	-
						1906.					
1422	Nov. 17	726	,,	37	R	Oct. 15	2199	5	Tzn.	12	- -
1549	Dec. 19	726	,,	37	RD	,,	2199	5	,,	12	- -
1554	,,	726	,,	37	RD	April 3	1785	20	Turn-bull	1	—
1561	,,	726	,,	37	R	Feb. 20	1508	10	Ord.	38	
1566	,,	726	,,	37	RD	,,	1506	10	,,	39	-
1624	,,	726	,,	37	R	April 3	1785	20	Turn-bull	1	-
	1906.										
1678	Jan. 11	1529	,,	59	R	Feb. 1	1540	10	Ord.	60	
1680	,,	1529	,,	59	R	,,	1540	20	,,	60	
1681	,,	726	,,	37	R	Feb. 8	1611	10	,,	40	-
1682	,,	726	,,	37	R	Feb. 1	1540	20	,,	60	-
1683	,,	726	,,	37	R	Feb. 8	1542	10	,,	61	-
1684	,,	1529	,,	59	R	,,	1493	10	,,	58	
1686	,,	726	,,	37	R	Feb. 1	1540	20	,,	60	
1688	,,	726	,,	37	R	Feb. 8	1529	10	,,	59	
1689	,,	1529	,,	59	R	,,	1529	10	,,	59	
1691	,,	726	,,	37	R	,,	1493	10	,,	58	
1692	,,	726	,,	37	R	,,	1471	10	,,	57	
1693	,,	1529	,,	59	R	Feb. 1	1540	10	,,	60	
1694	,,	726	,,	37	R	Feb. 8	1542	10	,,	61	
1696	,,	726	,,	37	R	,,	1611	10	,,	40	
1697	,,	726	,,	37	R	,,	1471	10	,,	57	-
1698	,,	726	,,	37	R	,,	1471	10	,,	57	
1699	,,	726	,,	37	R	,,	1493	10	,,	58	
1701	,,	1529	,,	59	R	,,	1542	10	,,	61	
1702	,,	1529	,,	59	R	Feb. 1	1540	10	,,	60	
1703	,,	726	,,	37	R	Feb. 8	1493	10	,,	58	
1704	,,	1529	,,	59	R	,,	1611	10	,,	40	
1706	,,	726	,,	37	R	,,	1529	10	,,	59	
1707	,,	726	,,	37	R	Feb. 1	1540	20	,,	40	
1711	,,	726	,,	37	R	Feb. 6	726	2	,,	37	
1713	,,	726	,,	37	R	,,	726	2	,,	37	—
1933	May 16	1863	,,	61	R	June 5	1785	10	Turn-bull	1	
1934	,,	1863	,,	61	R	,,	1785	10	,,	1	

R —Reaction. RD —Reaction with Dikkop.

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

TEST.						TEST.					
	Virus.						Virus.				
Date of Injection.	No.	Qu.	Orig.	Gen.	Result	Date of Injection.	No.	Qu.	Orig.	Gen.	Result.
		c.c.						c.c.			
1906.						1906.					
Mar. 14	1779	9000	Ord.	61		Sept. 20	1965	5	Tzn.	1	—
Mar. 6	1788	9000	Dale	1		,,	1965	5	,,	1	—
Oct. 15	2199	5	Tzn.	12	—						
April 3	1785	20	Turnbull	1	—						
Oct. 15	2199	5	Tzn.	12	—						
Oct. 15	2199	5	,,	12	—	Dec. 30	2477	9000	OTB LPW	1	—
Oct. 15	2199	5	,,	12							

Immunisation of Mules with Ordinary Virus.—(*cont.*)

Mule No.	Immunisation. Date of Injection.	Virus. No.	Orig.	Gen.	Result.	Test. Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
	1906.					1906.		c.c.			
1986	May 16.	1863	Ord.	61	R	June 5	1785	10	Turn-bull	1	—
1987	,,	1863	,,	61	R	,,	1785	10	,,	1	—
	1907.					1907.					
2525	Jan. 12	2287	,,	38	R	Feb. 21	2268	2	Tzu.	1	R
2528	,,	2287	,,	38	RD	,,	1954	2	Bul.	1	RD
2529	,,	2287	,,	38	RD	,,	2268	2	Tzu.	1	R
2532	,,	2287	,,	38	R	,,	1954	2	Bul.	1	RD
2566	Jan. 8	2407	,,	38	R	Jan. 22	2407	2	Ord.	38	—
2567	,,	2407	,,	38	?	,,	2407	2	,,	38	
2568	,,	2407	,,	38	R	,,	2407	2	,,	38	
2569	,,	2407	,,	38	R	,,	2407	2	,,	38	
2570	,,	2407	,,	38	R	,,	2407	2	,,	38	
2571	,,	2407	,,	38	R	,,	2407	2	,,	38	
2572	,,	2407	,,	38	R	,,	2407	2	,,	38	
2573	,,	2407	,,	38	R	,,	2407	2	,,	38	
2574	,,	2407	,,	38	RD	,,	2407	2	,,	38	
2575	,,	2407	,,	38	?	,,	2407	2	,,	38	
2576	,,	2407	,,	38	R	,,	2407	2	,,	38	
2577	,,	2407	,,	38	R	,,	2407	2	,,	38	—
	1905.					1906.					
1548	Dec. 19	1427	,,	38	R	Feb. 20	1506	10	,,	39	
1551	,,	1427	,,	38	R	Jan. 6	1611	20	,,	40	
	1906.										
1636	Jan. 11	1427	,,	38	R	Feb. 1	1540	20	,,	60	
1637	,,	1427	,,	38	R	Feb. 8	1529	10	,,	59	—
1685	,,	1427	,,	38	R	,,	1542	10	,,	61	
1695	,,	1427	,,	38	RD	,,	1471	10	,,	57	
1700	,,	1427	,,	38	R	Feb. 1	1540	10	,,	60	
	1905.					1906.					
1550	Dec. 19	1487	,,	1	R	Feb. 20	1506	10	,,	39	
1567	,,	1487	,,	1	R	Jan. 6	1611	20	,,	40	
1616	,,	*	,,	1	R	Feb. 20	726	10	,,	37	

R—Reaction. ? Doubtful. RD—Reaction with Dikkop.
RD† Reaction with Dikkop and Died.
* *N.B.*—Virus 1427, 1487. See note on page 91.

IMMUNISATION OF MULES WITH ORDINARY VIRUS.—(*cont.*)

Test.						Test.					
Date of Injection.	Virus.				Result.	Date of Injection.	Virus.				Result.
	No.	Qu.	Orig.	Gen.			No.	Qu.	Orig.	Gen.	
		c.c.									
1907.											
Mar. 13	1954	2	Bul.	1	R						
,,	2268	2	Tzo.	1	—						
,,	1954	2	Bul.	1							
1906.											
Feb. 20	1506	10	Ord.	39							
Feb. 20	1506	10	Ord.	39	—						

R Reaction.

Analysis from Preceding Tables.

295 mules immunised with Ordinary virus were hyperimmunised or tested as follows :—

37 mules, once hyperimmunised with O : No reaction.
41 ,, twice ,, ,, : ,,
13 ,, three times ,, ,, : ,,
4 ,, four ,, ,, ,, : ,,
2 ,, five ,, ,, ,, : ,,
15 ,, once hyperimmunised with O and tested with Tzaneen : No reaction.

50 mules, twice hyperimmunised with O and tested with Tzaneen : 1 reaction ; 3 reactions and dikkop ; 1 death.

8 mules, three times hyperimmunised with O and tested with Tzaneen : No reaction.

6 mules, four times hyperimmunised with O and tested with Tzaneen : 1 reaction.

1 mule, five times hyperimmunised with O and tested with Tzaneen : No reaction.

2 mules hyperimmunised with O : No reaction ; tested with Tzaneen : both reactions ; and thirdly, hyperimmunised with Tzaneen : no reaction.

1 mule, twice hyperimmunised with O, then tested with Tzaneen, gave no reaction.

2 mules, thrice hyperimmunised with O, then tested with Tzaneen and thirdly with Tzaneen : 1 mule had reaction and dikkop on the second test.

1 mule, twice hyperimmunised with O, and tested with Bulawayo, had reaction, dikkop, and died.

2 mules, hyperimmunised with O, then tested with Bulawayo, and thirdly hyperimmunised with Bulawayo : 1 mule had a reaction on the second test.

1 mule, twice hyperimmunised with O, then tested with Bulawayo, and thirdly hyperimmunised with Bulawayo, gave no reaction

2 mules, thrice hyperimmunised with O, then tested with Bulawayo, and thirdly hyperimmunised with Bulawayo: Both reaction and dikkop on the second test.

2 mules, twice hyperimmunised with O and tested with {Tzaneen / Bulawayo}: Both reacted.

2 mules, thrice hyperimmunised with O and tested with {Tz. / Bul.}: No reaction.

7 mules, first hyperimmunised with O, secondly tested with Tzaneen, and thirdly with Bulawayo: 1 mule died with the third test; 1 mule had reactions with the second and third test.

7 mules, twice hyperimmunised with O, secondly tested with Tzaneen, and thirdly with Bulawayo: 1 reaction with second test, 3 reactions and dikkop with the third test, and 1 death with the third test.

2 mules, thrice hyperimmunised with O, secondly tested with Tzaneen, and thirdly with Bulawayo: 1 reaction with the third test.

1 mule, hyperimmunised with O, secondly tested with Tzaneen, thirdly tested with Bulawayo, and fourthly hyperimmunised with Bulawayo, had a distinct reaction with the second and doubtful reaction with the third test.

2 mules, twice hyperimmunised with O, secondly tested with Tzaneen, thirdly with Bulawayo, and fourthly hyperimmunised with Bulawayo: 1 reaction with the second test.

1 mule, four times hyperimmunised with O, then tested with {Tz. / Bul.} and thirdly with Bulawayo, gave no reaction.

1 mule, four times hyperimmunised with O, secondly tested with Tzaneen, and thirdly with OTB: No reaction.

2 mules, twice hyperimmunised with O, secondly tested with Tzaneen, and thirdly hyperimmunised with OTBLPW: No reactions.

1 mule, four times hyperimmunised with O, secondly tested with Tzaneen, thirdly tested with Bulawayo, and fourthly hyperimmunised with OTBLPW : No reaction.

3 mules, first hyperimmunised with O, then hyperimmunised with {Ord. / Spont.} and thirdly tested with Tzaneen : No reaction.

12 mules, first hyperimmunised with O, then hyperimmunised with Spont. C., and thirdly tested with Tzaneen : No reactions.

1 mule, first hyperimmunised with O, then hyperimmunised with Spont. C., and thirdly tested with {Tz. / Bul.} : No reaction.

1 mule, hyperimmunised with O, secondly hyperimmunised with Spont. C., thirdly tested with Tzaneen, and fourthly hyperimmunised with Tzaneen : No reactions.

1 mule, hyperimmunised with O, secondly hyperimmunised with Spont. C., thirdly tested with Tzaneen, fourthly tested with Bulawayo, and fifthly hyperimmunised with Bulawayo, had a reaction with the fourth test.

36 mules were only tested with O and gave no reactions.

1 mule, first tested with O and secondly tested with Tzaneen No reaction.

1 mule, first tested with O and secondly tested with Spont. C. : No reaction.

1 mule, first tested with O secondly tested with Tzaneen, and thirdly with OTBLPW : No reaction.

7 mules were only tested with Tzaneen : 1 reaction.

1 mule, first test with Tzaneen and secondly hyperimmunised with Tzaneen : Reaction with the first test.

3 mules, first test Tzaneen and second test Bulawayo : 1 mule had a reaction with the first test ; 1 mule had a reaction with the second test ; 1 mule had reactions with both tests.

1 mule, first tested with Tzaneen, secondly with Bulawayo, and thirdly hyperimmunised with Bulawayo, had reactions with the first and second tests.

2 mules were hyperimmunised with Bulawayo and had reactions, dikkop, and died.

1 mule tested with Bulawayo had reaction, dikkop, and died.

1 mule, first test with Bulawayo and secondly with Tzaneen: Reaction and dikkop with the first test.

5 mules were tested with Spontaneous case: No reactions.

1 mule, first test with Spontaneous case and secondly with Tzaneen: No reaction.

7 mules immunised with virus donkey 1427 and tested with O: No reactions.

2 mules, immunised with mule virus 1487 and tested with O: No reactions.

1 mule, immunised with virus X and tested with O: No reaction.

Summary of Results of Tests on Mules Previously Immunised with Ordinary Virus.

No. of Mules.	Tested with.	Result.			
		Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Died.
273	Ordinary Virus	—	—	—	—
139	Tzaneen	12	4	—	1
36	Bulawayo	7	6	1	6
6	Tzaneen and Bulawayo ..	2	—	—	—
1	OTB	—	—	—	—
4	OTBLPW	—	—	—	—
22	Spontaneous Cases	—	—	—	—
3	Ord.—Spontaneous Cases ..	—	—	—	—

IMMUNISATION OF MULES WITH VIRUS OF SPONTANEOUS AND RELAPSE CASES.

Immunisation.						Test.					
Mule No.	Date of Injection.	Virus. No.	Orig.	Gen.	Result.	Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
	1906.					1906.		c.c.			
1805	April 26	1785	Turn-bull	1	R	May 12	1918	10	Turn-bull	4	—
1919	April 4	1785	,,	1	R	April 26	1785	20	,,	1	—
1920	April 26	1785	,,	1	R	May 12	1918	10	,,	4	—
1921	,,	1785	,,	1	R	,,	1918	10	,,	4	—
1922	,,	1785	,,	1	R	,,	1918	10	,,	4	—
1923	April 4	1785	,,	1	R	April 26	1785	20	,,	1	—
1924	April 26	1785	,,	1	?	May 12	1918	10	,,	4	—
1925	April 4	1785	,,	1	R	April 26	1785	20	,,	1	—
1927	,,	1785	,,	1	R	,,	1785	20	,,	1	—
1929	,,	1785	,,	1	R	,,	1785	20	,,	1	—
1931	,,	1785	,,	1	R	,,	1785	20	,,	1	—
1978	May 16	1785	,,	1	R	June 5	1785	10	,,	1	—
1979	,,	1785	,,	1	R	,,	1785	10	,,	1	—
1980	,,	1785	,,	1	R	,,	1785	10	,,	1	—
1982	,,	1785	,,	1	R	,,	1785	10	,,	1	—
1983	,,	1785	,,	1	?	,,	1785	10	,,	1	—
1984	,,	1785	,,	1	R	,,	1785	10	,,	1	—
1985	,,	1785	,,	1	R	,,	1785	10	,,	1	—
1788	Feb. 25	Dale	Potchefstr'm		R	Oct. 15	2199	5	Tzn.	12	—
1926	April 18	1788	Dale	1	R	April 26	1785	20	Turn-bull	1	—
1932	June 2	Edgar	Pietersburg		R	Dec. 29	2476	9000	OTB	1	R
1894	May 14	1957	Warm baths	1	R	June 2	P.W.D	5	—	—	R
2284	Oct. 19	1418	Cape Colony	1	R	Nov. 27	1938	10	Ord.	62	—
							2199	10	Tzn.	12	—
							1964	10	Bul.	2	—
						1907.					
2455	Dec. 5	1418	,,	1	R	Jan. 8	2298	2	,,	11	R†
2456	,,	2284	,,	1	R	,,	2199	2	Tzn.	12	R†
2463	Dec. 19	1418	,,	1	?	,,	1938	2	Ord.	62	R†

R—Reaction. ?—Doubtful. R†—Reaction and died.

ANALYSIS FROM PRECEDING TABLE.

25 mules immunised with virus Spontaneous cases were tested as follows :—

17 mules were tested with virus of Spontaneous case : No reactions.

2 mules, tested first with Spont. and secondly with Tzaneen : No reactions.

1 mule was tested with OTB and had a reaction.

1 mule, first test with Tzaneen and second test with OTB : No reaction.

1 mule tested with Ordinary had reaction, dikkop, and died.

1 ,, ,, Tzaneen ,, ,, ,,

1 ,, ,, Bulawayo ,, ,, ,,

1 mule, first test $\left\{\begin{matrix}O\\T\\B\end{matrix}\right.$, second test $\left\{\begin{matrix}O\\T\\B\end{matrix}\right.$, and third test Ord. : No reaction.

1 mule immunised with virus Warmbaths was tested with virus of a Public Works Department mule and showed a distinct reaction. A subsequent test with Tzaneen gave no reaction.

Immunisation of Mules with Virus of Spontaneous and Relapse Cases.

Test.							Test.					
Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.		Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
1906.		c.c.							c.c.			
Oct. 15	2199	5	Tzn.	12	—							
Oct. 15	2199	5	Tzn.	12	—							
Dec. 29	2476	9000	OTB	1	—							
Oct. 15	2199	5	Tzn.	12	—							
Dec. 7	1938	7	Ord.	62	—		1907. Jan. 1	2287	2	Ord.	38	—
	2199	7	Tzn.	12	—							
	2298	6	Bul.	11	—							

Summary of Results of Tests on Mules Previously Immunised with Virus obtained from Spontaneous and Relapse Cases.

No. of Mules.	Tested with.	Result.			
		Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Died.
2	Ordinary Virus	—	—	—	1
4	Tzaneen	—	—	—	1
1	Bulawayo	—	—	—	1
1	O-T-B-	—	—	—	—
2	OTB	1	—	—	—
19	Spontaneous Cases	—	—	—	—

Immunisation of Horses with Ordinary Virus.

Horse No.	Immunisation. Date of Injection.	Serum. Orig.	Virus. No.	Virus. Orig.	Virus. Gen.	Result.	Test. Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.
	1902.						1902.		c.c.			
160	Sept. 9	Ord.	138	Ord.	5	—	Oct. 10	138	10	Ord.	5	—
	Oct. 31	,,	102	,,	4	—						
							1903.					
	Nov. 20	,,	166	,,	5	—	Feb. 9	262	100	,,	5	—
	Dec. 5	,,	216	,,	6	—	Feb. 20	265	200	,,	6	—
							Mar. 5	278	500	,,	7	—
							April 8	295	1000	,,	9	—
							1902.					
172	Oct. 10	,,	153	,,	5	—	Dec. 1	174	200	,,	5	—
	Oct. 24	,,	102	,,	4	—						
	Nov. 13	,,	166	,,	5	—						
	1905.						1905.					
290	Mar. 13	,,	726	,,	37	RD	April 10	697	8250	,,	42	—
							April 25	1053	1500	,,	44	—
							1906.					
							Jan. 21	1646	9000	,,	61	—
	1903.						1903.					
301	May 1	,,	295	,,	9	R	Aug. 17	354	500	,,	12	—
							Sept. 14	370	1000	,,	14	—
	1906.						1906.					
358	April 25	,,	1863	,,	61	RD	June 15	2038	9000	,,	4	—
	1906.						1906.					
384	June 30	,,	1863	,,	61	R	July 19	2060	20	Bul.	2	R†
	1904.						1904.					
398	Nov. 4	,,	547	,,	32	RD	Dec. 11	668	2750	Ord.	35	—
							Dec. 30	666	4000	,,	36	—
407	,,	,,	547	,,	32	R	Dec. 9	668	1500	,,	35	—
							Dec. 29	671	950	,,	37	—
473	,,	,,	547	,,	32	R	Dec. 13	658	6000	,,	35	—
	1904.						1905.					
545	Dec. 18	,,	658	,,	35	R	Feb. 6	712	7000	,,	39	—
							Feb. 9	747	1500	,,	39	—
							1906.					
							July 21	2058	10	Tzn.	6	—
							Sept. 1	1964	5	Bul.	2	—
	1904.						1905.					
602	Dec. 2	,,	659	,,	35	RD	May 10	1053	6500	Ord.	44	—
							,,	1099	3000	,,	46	—
							1906.					
							Jan. 11	1632	3000	,,	61	—
							Jan. 20	1646	6000	,,	61	—

R—Reaction. RD—Reaction with Dikkop. R†—Reaction and died.

Immunisation of Horses with Ordinary Virus.

Test.						Test.					
Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.	Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.
1903.		c.c.				1904.		c.c.			
May 5	300	1500	Ord.	10	—	Jan. 12	411	2000	Ord.	18	—
June 3	316	2500	,,	11	—						
July 21	335	1700	,,	11	—	Mar. 30	487	500	,,	23	—
Aug. 11	338	2000	,,	12	—						
						1903.					
Feb. 9	262	400	,,	5	—	Sept. 12	352	2000	,,	14	—
Feb. 20	265	1000	,,	6	—						
Mar. 4	279	2000	,,	7	—						
						1904.					
May 3	300	500	,,	10	—	Jan. 12	411	2000	,,	18	—
May 21	292	1000	,,	11	—	Mar. 29	487	1500	,,	23	—
1905.						1905.					
July 25	1246	9000	,,	43	—	Dec. 20	1540	9000	,,	60	—
1906.						1907.					
June 22	2032	9000	Tzn.	3	RD	Feb. 6	2624	6000	,,	66	—
1903.						1904.					
Dec. 30	410	1500	Ord.	17	—	Feb. 2	425	2000	,,	20	—
						Nov. 21	640	5	,,	33	—
1905.						1906.					
July 28	1249	6000	,,	42	—	Jan. 10	1632	6000	,,	61	—
						Jan. 11	1589	2500	,,	61	—
July 31	1248	3000	,,	43	—						
						Mar. 20	1890	9000	Turn-bull	2	—
1906.											
Aug. 2	1869	20	Tzn.	1	—	Mar. 29	1856	6000	,,	3	—
Nov. 14	2401	9000	OTB	3	—	Mar. 31	1876	3000	Elder	1	—
						Aug. 17	1964	1	Bul.	2	—
1905.						1905.					
Aug. 1	1248	9000	Ord.	43	—	Dec. 6	1493	9000	Ord.	58	—
1906.						1907.					
July 4	2033	9000	Tzn.	5	RD	Feb. 13	2634	6000	,,	67	—

RD—Reaction with Dikkop.

IMMUNISATION OF HORSES WITH ORDINARY VIRUS.—(*cont.*)

Horse No.	Immunisation. Date of Injection.	Serum. Orig.	Virus. No.	Virus. Orig.	Virus. Gen.	Result.	Test. Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.
	1904.						1904.		c.c.			
611	Oct. 11	Ord.	623	Ord.	31	R	Nov. 17	640	4500	Ord.	33	—
612	Oct. 1	,,	561	,,	30	RD	,,	640	4500	,,	33	—
615	Oct. 20	,,	547	,,	32	R	Dec. 13	658	5000	,,	35	—
							1905. Feb. 7	753	3000	,,	39	—
							1906. Jan. 12	1589	2000	,,	61	—
							,,	1538	6000	,,	40	—
							1905.					
624	Dec. 30	,,	694	,,	36	RD	Feb. 6	753	4500	,,	39	—
							Feb. 8	747	3500	,,	39	—
	1905.											
714	Mar. 13	,,	726	,,	37	R	April 11	808	9000	,,	42	—
719	June 10	,,	726	,,	37	R	July 9	1210	6000	,,	40	—
							July 24	1246	3000	,,	43	—
							1906. May 18	1961	6000	Turn-bull	6	—
							May 19	1962	3000	,,	6	—
							1905.					
720	April 17	,,	726	,,	37	R	Oct. 18	1358	9000	Ord.	51	—
731	Mar. 13	,,	726	,,	37	RD	April 11	808	6000	,,	42	—
							,,	865	1500	,,	42	—
							1906. Jan. 11	1589	9000	,,	61	—
							Aug. 29	2111	9000	Bul.	7	—
							1905.					
811	Mar. 13	,,	726	,,	37	RD	April 10	808	4000	Ord.	42	—
							April 11	866	3000	,,	42	—
							1906. Jan. 10	1632	6000	,,	61	—
							Jan. 12	1538	3000	,,	40	—
							1905.					
812	,,	,,	726	,,	37	R	April 11	865	9000	,,	42	—
							1906. Jan. 11	1632	9000	,,	61	—
							Aug. 17	1964	1	Bul.	2	—
							Dec. 16	2416	9000	OTB LPW	2	—
							1905.					
868	Feb. 28	,,	726	,,	37	RD	April 11	866	8000	Ord.	42	—
869	April 17	,,	726	,,	37	RD	May 16	1099	9000	,,	46	—
							1906. Jan. 12	1538	9000	,,	40	—

R—Reaction. RD—Reaction with Dikkop.

IMMUNISATION OF HORSES WITH ORDINARY VIRUS.—(*cont.*)

Test.						Test.					
Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.	Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.
1905.		c.c.									
Feb. 7	712	4500	Ord.	39	—						
,,	712	4500	,,	39							
						1905.		c.c.			
July 28	1249	6000	,,	42	—	Nov. 30	1471	8500	Ord.	57	—
July 31	1248	3000	,,	13							
1906.						1907.					
March 29	1856	6000	Turnbull.	3		Feb. 6	2626	6000	,,	66	—
March 31	1276	3000	Ord.	45	—						
1905.											
July 18	1219	5500	,,	42	—						
July 24	1247	3500	,,	43	—						
						1906.					
Oct. 28	1363	9000	,,	53	—	Jan. 28	1541	9000	,,	62	—
1907.											
Feb. 6	2625	3000	,,	66	—						
1905.						1905.					
July 18	1219	5500	,,	42	—	Nov. 9	1445	6000	,,	55	
July 21	1242	3500	,,	41	—	Nov. 22	1494	3000	,,	57	—
1906.						1906.					
June 15	2038	9000	,,	4	—	July 21	2060	10	Bul.	2	—
Dec. 20	2480	9000	OTB	3	—						
1905.			LPW			1905.					
July 25	1247	9000	Ord.	43	—	Nov. 15	1446	9000	Ord.	56	—
1906.						1906.					
April 1	1876	6000	Elder	1	—	July 2	2060	10	Bul.	2	R
April 6	1891	3000	,,	2	—						
1905.						1905.					
July 25	1247	9000	Ord.	43	—	Nov. 23	1494	9000	Ord.	57	—
1906.						1906.					
April 1	1876	6000	Elder	1	—	Aug. 3	1869	1	Tzn.	1	—
April 6	1891	3000	,,	2	—						
Sept. 5	1964	5	Bul.	2	—	Oct. 2	2298	6000	Bul.	11	—
						Oct. 9	2150	3000	,,	12	—
1905.						1905.					
Aug. 31	1248	9000	Ord.	43	—	Dec. 6	1493	9000	Ord.	58	—
1906.											
July 21	2060	16	Bul.	2	RD						

R—Reaction. RD—Reaction with Dikkop.

IMMUNISATION OF HORSES WITH ORDINARY VIRUS.—(*cont.*)

Horse No.	Immunisation. Date of Injection.	Immunisation. Serum Orig.	Immunisation. Virus. No.	Immunisation. Virus. Orig.	Immunisation. Virus. Gen.	Immunisation. Result.	Test. Date of Injection.	Test. Virus. No.	Test. Virus. Qu.	Test. Virus. Orig.	Test. Virus. Gen.	Test. Result.
875	1905. Mar. 13	Ord.	726	Ord.	37	RD	1905. April 11	866	c.c. 9000	Ord.	42	—
							1906. Feb. 24	1854	3000	,,	64	—
							Feb. 26	1853	6000	,,	64	—
							Sept. 6	2151	8500	Tzn.	10	—
877	,,	,,	726	,,	37	RD	1905. May 29	1090	7500	Ord.	47	—
							June 5	733	2000	,,	48	—
							1906. April 6	1880	6000	Turn-bull	3	—
							April 8	1891	3000	Elder	2	—
881	,,	,,	726	,,	37	RD	1905. April 10	697	1750	Ord.	42	—
							May 29	1090	2250	,,	47	—
							June 5	733	5500	,,	47	—
							1906. Mar. 21	1889	6000	Turn-bull	2	—
							Mar. 23	1892	3000	,,	2	—
							Aug. 17	1964	10	Bul.	2	—
882	,,	,,	726	,,	37	RD	1905. April 10	697	9000	Ord.	42	—
							1906. Jan. 11	1589	8500	,,	61	—
1055	April 17	,,	726	,,	37	R	1905. May 16	1095	9000	,,	46	—
1056	,,	,,	726	,,	37	R	,,	1095	9000	,,	46	—
							1906. Feb. 27	1854	6000	,,	64	—
							Mar. 6	1862	3000	,,	60	—
1075	May 15	,,	726	,,	37	RD	1905. June 14	944	9000	,,	49	—
1076	,,	,,	726	,,	37	RD	,,	809	9000	,,	49	—
							1906. April 7	1891	9000	Elder	2	—
1077	,,	,,	726	,,	37	RD	1905. June 14	716	9000	Ord.	49	—
1078	,,	,,	726	,,	37	RD	,,	809	9000	,,	49	—
							1906. April 8	1891	9000	Elder	2	—

R—Reaction. RD—Reaction with Dikkop.

Immunisation of Horses with Ordinary Virus.—(*cont.*)

Test.					
Date of Injection.	Virus.				Result.
	No.	Qu.	Orig.	Gen.	
1905.		c.c.			
July 18	1209	3000	Ord.	42	
July 24	1246	6000	,,	43	
1906.					
June 15	2038	9000	,,	4	
1907.					
Feb. 12	2632	3000	,,	67	
1905.					
Sept. 5	1301	6000	,,	46	
Sept. 6	1302	3000	,,	46	
1906.					
July 4	2056	9500	Tzn.	5	
1905.					
Sept. 6	1301	6000	Ord.	46	—
,,	1302	3000	,,	46	—
1906.					
June 15	1916	9600	,,	45	—
Aug. 29	2111	9000	Bul.	7	—
1905.					
July 24	1247	3000	Ord.	43	
July 25	1246	6000	,,	43	
1906.					
April 1	1876	6000	Elder	1	—
April 6	1891	3000	,,	2	—
1905.					
Aug. 22	1255	9000	Ord.	45	—
Aug. 23	1255	9000	,,	45	—
1906.					
July 12	2058	8500	Tzn.	6	—
1905.					
Sept. 5	1302	3000	Ord.	46	—
Sept. 6	1301	6000	,,	46	—
1906.					
July 12	2059	9000	Tzn.	6	RD
1905.					
Sept. 6	1302	9000	Ord.	46	—
,,	1302	3000	,,	46	—
Sept. 8	1316	6000	,,	47	—
1906.					
July 12	2059	9000	Tzn.	6	—

Test.					
Date of Injection.	Virus.				Result.
	No.	Qu.	Orig.	Gen.	
1905.		c.c.			
Nov. 23	1494	9000	Ord.	57	—
1906.					
July 21	2058	10	Tzn.	6	—
Jan. 11	1643	3000	Ord.	61	—
Jan. 17	1542	6000	,,	61	—
1907.					
Feb. 14	2635	2500	,,	68	—
1906.					
Jan. 11	1643	9000	,,	61	—
Aug. 3	1964	1	Bul.	2	—
Dec. 12	2416	9000	OTB LPW	2	—
1905.					
Nov. 30	1471	9000	Ord.	57	—
1907.					
Feb. 15	2635	2000	,,	68	RD
Feb. 19	2637	1000	,,	69	RD
1905.					
Dec. 12	1528	9000	,,	59	—
,,	1528	9000	,,	59	—
1907.					
Feb. 11	2630	3000	,,	67	—
1906.					
Jan. 11	1643	9000	,,	61	—
1907.					
Feb. 12	2632	6000	,,	67	—
1906.					
Jan. 17	1542	9000	,,	61	—
1907.					
Feb. 12	2632	6000	,,	67	—

RD—Reaction with Dikkop.

IMMUNISATION OF HORSES WITH ORDINARY VIRUS.—(*cont.*)

Horse No.	Immunisation. Date of Injection.	Immunisation. Serum. Orig.	Immunisation. Virus. No.	Immunisation. Virus. Orig.	Immunisation. Virus. Gen.	Immunisation. Result.	Test. Date of Injection.	Test. Virus. No.	Test. Virus. Qu.	Test. Virus. Orig.	Test. Virus. Gen.	Test. Result.
	1905.						1905.		c.c.			
1081	May 15	Ord.	726	Ord.	37	R	June 13	716	2500	Ord.	49	—
							June 23	943	6500	,,	50	—
							1906.					
							May 9	1937	6000	Turn-bull	6	—
							May 17	1961	3000	,,	7	—
							1905.					
1082	,,	,,	726	,,	37	R	June 14	1063	9000	Ord.	49	—
							1906.					
							May 10	1918	9000	Turn-bull	6	—
							1905.					
1084	,,	,,	726	,,	37	RD	June 14	716	9000	Ord.	49	—
1085	,,	,,	726	,,	37	R	June 15	1063	3000	,,	49	—
							June 22	943	6000	,,	50	—
							1906.					
							Aug. 17	1964	1	Bul.	2	—
							Nov. 20	2402	9000	OTB	4	—
							1905.					
1086	,,	,,	726	,,	37	RD	June 14	944	9000	Ord.	49	—
							1906.					
							May 10	1938	9000	,,	62	—
							1907.					
							Jan. 26	2556	9000	Spont. + OTB LPW	1	—
							1905.					
1087	,,	,,	726	,,	37	RD	June 20	1100	8500	Ord.	50	—
1088	,,	,,	726	,,	37	RD	June 15	1063	3000	,,	49	—
							June 22	943	5500	,,	50	—
							1906.					
							May 10	1918	6000	Turn-bull	6	—
							May 17	1961	3000	,,	7	—
							1905.					
1089	,,	,,	726	,,	37	RD	June 13	944	2000	Ord.	49	—
							June 14	1063	6500	,,	49	—
1092	,,	,,	726	,,	37	RD	June 16	716	2500	,,	49	—
							June 23	943	6000	,,	50	—
							1906.					
							May 11	1937	9000	Turn-bull	4	—
							Aug. 16	2086	8000	Bul.	5	—

R—Reaction. RD—Reaction with Dikkop.

IMMUNISATION OF HORSES WITH ORDINARY VIRUS.—(*cont.*)

TEST.						TEST.					
Date of Injection.	Virus.				Result.	Date of Injection.	Virus.				Result.
	No.	Qu.	Orig.	Gen.			No.	Qu.	Orig.	Gen.	
1905.		c.c.				1906.		c.c.			
Sept. 23	1355	3000	Ord.	48	—	Jan. 28	1526	9000	Ord.	62	—
Sept. 26	1349	6000	,,	49	—						
1906.											
July 12	2058	8500	Tzn.	6	RD†						
1905.											
Sept. 6	1302	3000	Ord.	46	—	Jan. 11	1643	9000	,,	61	—
Sept. 8	1310	6000	,,	47	—						
1906.											
July 20	1959	9000	Bul.	3	—	Nov. 22	2403	6000	OTB	4	—
						Dec. 5	2418	3000	,,	5	—
July 21	2058	10	Tzn.	6	—	Aug. 2	1869	20	Tzn.	1	—
Sept. 1	1964	5	Bul.	2	—	Oct. 2	2298	9000	Bul.	11	?
1905.											
Sept. 9	1310	9000	Ord.	47	—	Jan. 17	1542	6000	Ord.	61	—
						Jan. 19	1588	3000	,,	61	—
1906.											
July 20	1959	8500	Bul.	3	RD	Dec. 20	2480	9000	OTB LPW	3	—
1905.											
Sept. 27	1349	9000	Ord.	49	—	Jan. 29	1527	3000	Ord.	40	—
						Jan. 31	1644	6000	,,	40	—
1906.											
July 21	2058	10	Tzn.	6	R†						
1905.											
Sept. 9	1310	9000	Ord.	47	—						
Sept. 27	1356	9000	,,	49	—	Jan. 10	1538	6000	,,	40	—
						Jan. 19	1588	3000	,,	61	—
1906.											
July 21	2060	10	Bul.	2	—	Aug. 2	1964	20	Bul.	2	—
Dec. 5	2418	9000	OTB	5	—						

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop.
RD†—Reaction with Dikkop and died.

IMMUNISATION OF HORSES WITH ORDINARY VIRUS.—(*cont.*)

Horse No.	Immunisation.						Result.	Test.						Result.
	Date of Injection.	Serum.	Virus.					Date of Injection.	Virus.					
		Orig.	No.	Orig.	Gen.				No.	Qu.	Orig.	Gen.		
										c.c.				
1094	1905. May 15	Ord.	726	Ord.	37	RD		1905. June 14	1063	9000	Ord.	49		—
								1906. May 18	1961	9000	Turn-bull	6		—
								1905.						
1098	,,	,,	726	,,	37	R		June 19	1100	3750	Ord.	50		—
								June 22	943	5000	,,	50		—
1162	June 10	,,	726	,,	37	RD		July 3	1159	6000	,,	39		—
								July 9	1210	3000	,,	40		—
								1906. May 18	1962	9000	Turn-bull	6		—
								1905.						
1163	,,	,,	726	,,	37	RD		July 9	1210	4500	Ord.	40		?
								July 13	1218	3000	,,	41		?
								July 17	1209	1500	,,	42		?
								1906. May 18	1962	9000	Turn-bull	6		—
								1905.						
1170	,,	,,	726	,,	37	RD		July 13	1218	3000	Ord.	41		—
								July 18	1219	5500	,,	42		—
								1906. May 19	1962	6500	Turn-bull	7		?
								May 23	1960	2500	,,	8		?
								1905.						
1171	,,	,,	726	,,	37	RD		July 18	1209	8500	Ord.	42		—
								1906. May 19	1961	9000	Turn-bull	6		—
								1905.						
1172	,,	,,	726	,,	37	RD		July 18	1209	9000	Ord.	42		—
								1906. May 19	1962	4500	Turn-bull	7		—
								May 23	1974	4500	,,	8		—
								1905.						
1173	,,	,,	726	,,	37	RD		July 18	1209	9000	Ord.	42		—
1174	,,	,,	726	,,	37	RD		July 17	1217	1500	,,	41		—
								July 17	1242	5500	,,	41		—
								July 24	1246	1500	,,	43		—

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop.

IMMUNISATION OF HORSES WITH ORDINARY VIRUS.—(*cont.*)

Test.						Test.					
Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.	Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.
1905.		c.c.				1906.		c.c.			
Sept. 19	1311	3000	Ord.	48	—	Jan. 10	1538	3000	Ord.	40	—
Sept. 23	1355	6000	,,	48	—	Jan. 20	1588	6000	,,	61	—
Oct. 27	1363	6000	,,	53	—	Jan. 31	1644	3000	,,	40	—
Oct. 31	1382	3000	,,	54	—	Feb. 22	1851	6000	,,	39	—
1906.						1907.					
July 19	2014	3000	Tzn.	7	—	Feb. 6	2624	6000	,,	66	—
Aug. 24	2090	6000	,,	9	—						
1905.						1906.					
Oct. 27	1363	6000	Ord.	53	—	Jan. 31	1644	3000	,,	40	—
Oct. 31	1382	3000	,,	54	—	Feb. 22	1852	6000	,,	39	—
1906.						1907.					
July 19	2013	3000	Tzn.	7	R	Feb. 5	2624	6000	,,	66	—
Aug. 24	2090	6000	,,	9	R						
1905.						1906.					
Oct. 31	1382	6000	Ord.	54	—	Feb. 1	1644	9000	,,	40	—
Nov. 8	1445	3000	,,	55	—						
1907.											
Jan. 29	2553	9000	,,	65	—						
1905.											
Oct. 31	1382	6000	,,	54	—	Jan. 28	1584	3000	,,	62	—
Nov. 8	1445	3000	,,	55	—	Jan. 29	1527	6000	,,	62	—
Nov. 9	1445	6000	,,	55	—	Feb. 22	1852	3000	,,	39	—
Nov. 21	1494	3000	,,	57	—	Feb. 23	1855	6000	,,	40	—
1907.											
Feb. 7	2625	3000	,,	66	—						
1905.											
Nov. 9	1445	6000	,,	55	—						
Nov. 21	1494	3000	,,	57	—						

R—Reaction.

Immunisation of Horses with Ordinary Virus.—(*cont.*)

Horse No.	Immunisation. Date of Injection.	Serum. Orig.	Virus. No.	Virus. Orig.	Virus. Gen.	Result.	Test. Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.
	1905.						1905.		c.c.			
1175	June 10	Ord.	726	Ord.	37	RD	July 17	1217	6000	Ord.	41	—
							July 21	1242	3000	,,	41	—
							1906.					
							May 23	1974	9000	Turn-bull	8	—
							Dec. 5	2418	9000	OTB	5	—
							1905.					
1216	July 14	,,	726	,,	37	RD	Aug. 15	1259	9000	Ord.	44	—
							1906.					
							June 7	2006	9000	Elder	3	—
							1905.					
1220	July 14	,,	726	,,	37	R	Aug. 16	1259	9000	Ord.	44	—
1224	,,	,,	726	,,	37	RD	,,	1260	9000	,,	44	—
232	,,	,,	726	,,	37	R	,,	1260	9000	,,	44	—
236	Aug. 13	,,	726	,,	37	R	Sept. 23	1311	3000	,,	48	—
							,,	1355	6000	,,	48	—
							1906.					
							June 15	1916	8500	,,	45	—
							Sept. 3	1964	5	Bul.	2	—
							1905.					
238	Aug. 17	,,	726	,,	37	R	Sept. 12	1284	9000	Ord.	47	—
							1906.					
							June 22	2027	9000	Tzn.	3	RD†
239	July 14	,,	726	,,	37	RD	1905.					
							Aug. 23	1255	1500	Ord.	45	—
							Aug. 25	1264	7500	,,	44	—
							1906.					
							June 16	2028	3000	Tzn.	4	R
							June 22	2032	6000	,,	3	R
							1905.					
251	Aug. 3	,,	726	,,	37	R	Aug. 27	1264	6000	Ord.	44	?
							Aug. 30	1299	3000	,,	46	?
							1906.					
							June 22	2032	3000	Tzn.	3	RD
							June 23	2029	6000	Elder	4	RD
							1905.					
253	,,	,,	726	,,	37	RD	Aug. 26	1264	6000	Ord.	44	—
							Aug. 30	1299	3000	,,	46	—
							1906.					
							June 23	2027	6000	Tzn.	3	RD
							June 28	2026	3000	Elder	5	RD
							1905.					
258	,,	,,	726	,,	37	R	Aug. 30	1299	6000	Ord.	46	—
							Sept. 5	1301	3000	,,	46	—
							1907.					
							Feb. 20	2637	6000	,,	69	—

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop.
RD†—Reaction with Dikkop and died.

IMMUNISATION OF HORSES WITH ORDINARY VIRUS.—(*cont.*)

Test.						Test.					
Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.	Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
1905.		c.c.				1906.		c.c.			
Nov. 15	1446	9000	Ord.	56	—	Feb. 27	1854	9000	Ord.	64	?
1906.											
Aug. 3	1964	1	Bul.	2	RD	Aug. 15	2086	6000	Bul.	5	—
						Aug. 23	2093	3000	,,	6	—
1905.											
Dec. 12	1528	9000	Ord.	59	—	Mar. 19	1889	3000	Turn-bull	2	—
						Mar. 20	1890	6000	,,	2	—
1906.											
July 21	2058	10	Tzn.	6	RD†						
1905.											
Dec. 6	1493	4500	Ord.	58	—						
Dec. 18	1540	3000	,,	60	—						
Dec. 31	1504	9000	,,	40	—	Mar. 21	1890	9000	,,	2	—
1906.											
Aug. 3	1869	1	Tzn.	1	—	Aug. 17	1964	1	Bul.	2	—
Dec. 6	2406	9000	OTB LPW	1	R						
1905.											
Dec. 31	1631	9000	Ord.	40	—	Mar. 21	1889	9000	Turn-bull	2	—
Dec. 12	1529	9000	,,	59	—	Mar. 20	1890	6000	Turn-bull	2	—
						Mar. 21	1889	3000	,,	2	—
Dec. 12	1528	6000	,,	59	—	Mar. 15	1863	9000	Ord.	61	—
Dec. 18	1540	3000	,,	60	—						
1907.											
Feb. 6	2625	6000	,,	66	—						
1905.											
Dec. 12	1529	6000	,,	59	—	Mar. 21	1398	9000	Alten-roxel	1	R
Dec. 18	1543	3000	,,	60	—						
1907.											
Mar. 5	Relapse of dikkop										
1905.											
Dec. 19	889	9000	Ord.	6	—	June 29	2026	9000	Elder	5	R

R—Reaction ?—Doubtful. RD—Reaction with Dikkop.
RD†—Reaction with Dikkop and died.

IMMUNISATION OF HORSES WITH ORDINARY VIRUS.—(*cont.*)

Horse No.	Immunisation.						Test.					
	Date of Injection.	Serum.	Virus.			Result.	Date of Injection.	Virus.				Result.
		Orig.	No.	Orig.	Gen.			No.	Qu.	Orig.	Gen.	
1261	1905. Aug. 3	Ord.	726	Ord.	37	R	1905. Aug. 30	1299	c.c. 6000	Ord.	46	—
							Sept. 5	1301	3000	,,	46	—
							1906. July 4	2056	9000	Tzn.	5	—
1263	Aug. 17	,,	726	,,	37	RD	1905. Sept. 12	1284	9000	Ord.	47	—
1265	,,	,,	726	,,	37	RD	,,	1308	9000	,,	47	—
1283	,,	,,	726	,,	37	RD	,,	1308	8500	,,	47	—
1285	,,	,,	726	,,	37	RD	,,	1284	6000	,,	47	—
							Sept. 17	1348	3000	,,	48	—
							1906. July 5	2056	8000	Tzn.	5	—
1288	,,	,,	726	,,	37	RD	1905. Sept. 12	1308	2500	Ord.	47	—
							Sept. 18	1348	6500	,,	48	—
							Sept. 19	1311	1000	,,	48	—
							1906. July 4	2033	9000	Tzn.	5	R
1290	Sept. 9	,,	726	,,	37	R	1905. Oct. 4	1357	6000	Ord.	50	—
							Oct. 5	1359	3000	,,	50	—
1293	Aug. 17	,,	726	,,	37	R	Sept. 12	1284	3000	,,	47	—
							Sept. 18	1348	3000	,,	48	—
							Sept. 19	1311	3000	,,	48	—
							1906. July 21	2060	10	Bul.	2	R
1321	Sept. 9	,,	726	,,	37	RD	1905. Oct. 6	1359	8000	Ord.	50	—
							1906. July 5	2033	9000	Tzn.	5	R†
1322	,,	,,	726	,,	37	R	1905. Oct. 5	1359	6000	Ord.	50	—
							Oct. 17	1358	3000	,,	51	—
1350	,,	,,	726	,,	37	RD	Oct. 4	1357	3000	,,	50	—
							Oct. 5	1359	6000	,,	50	—

R—Reaction. RD—Reaction with Dikkop. R†—Reaction and died.

IMMUNISATION OF HORSES WITH ORDINARY VIRUS.—*(cont.)*

Test.						Test.					
Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.	Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
1905. Dec. 31	1504	c.c. 9000	Ord.	40	—	1906. Mar. 15	1865	c.c. 3000	Dale	2	—
						Mar. 20	1889	6000	Turnbull	2	—
1907. Feb. 14	2636	3000	,,	68	—						
1905. Dec. 20	1540	9000	,,	60	—	Mar. 15	1865	3000	Dale	2	—
						Mar. 20	1398	6000	Altenroxel	1	—
Dec. 19	1543	3000	,,	60							
Dec. 30	1631	6000	,,	40	—						
						1907.					
Dec. 19	1540	6000	,,	60		Feb. 6	2625	6000	Ord.	66	—
Dec. 29	1631	3000	,,	40	—						
						1906.					
Dec. 19	1543	3000	,,	60		Mar. 29	1645	6000	Turnbull	3	—
Dec. 30	1631	6000	,,	40	—						
						April 6	1891	3000	Elder	2	-
Dec. 31	1504	9000	,,	40	—	Mar. 24	1892	6000	Turnbull	2	—
						Mar. 28	1645	3000	,,	3	—
Dec. 31	1631	3000	,,	40	—	Mar. 29	1645	3000	,,	3	—
1906. Jan. 18	1542	6000	,,	61	—	April 7	1891	6000	Elder	2	
Oct. 30	2357	7500	OTB	2	—						
Feb. 21	1851	9000	Ord.	39	—	May 23	1974	9000	Turnbull	8	—
Feb. 22	1852	9000	,,	39	—						
Feb. 22	1851	3000	,,	39	—						
Feb. 23	1855	6000	,,	40	—						

IMMUNISATION OF HORSES WITH ORDINARY VIRUS.—(*cont.*)

Horse No.	Immunisation. Date of Injection.	Serum. Orig.	Virus. No.	Virus. Orig.	Virus. Gen.	Result.	Test. Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.
1351	1905. Sept. 9	Ord.	726	Ord	37	?	1905. Oct. 4	1357	c.c. 9000	Ord.	50	—
1352	,,	,,	726	,,	37	RD	Oct. 6	1359	9000	,,	50	—
1353	,,	,,	726	,,	37	R	Sept. 27	1349	6000	,,	49	—
							Oct. 3	1357	3000	,,	50	—
							1906. July 21	2058	10	Tzn.	6	—
1380	Oct. 10	,,	726	,,	37	R	1905. Nov. 22	1494	5500	Ord.	57	—
							Nov. 28	1471	3000	,,	57	—
1381	,,	,,	726	,,	37	R	Nov. 29	1471	9000	,,	57	—
							1907. Feb. 6	2626	6000	,,	66	—
1397	1906. June 30	,,	1863	,,	61	R	1906. July 19	2058	20	Tzn.	6	R†
1403	July 12	,,	1863	,,	61	RD	Aug. 17	1964	1	Bul.	2	RD
1448	Jan. 6	,,	726	,,	37	R	Feb. 21	1852	9000	Ord.	39	—
							Aug. 10	2082	9000	Tzn.	8	R
1453	Jan. 31	,,	726	,,	37	R	Mar. 15	1863	9000	Ord.	61	—
1515	1905. Dec. 19	,,	726	,,	37	R	Jan. 21	1588	3000	,,	61	—
							Jan. 27	1541	5500	,,	62	—
1579	1906. Jan. 6	,,	726	,,	37	?	Feb. 21	1852	9000	,,	39	—
							Nov. 20	2402	6000	OTB	4	—
							Nov. 21	2403	3000	,,	4	—
1587	,,	,,	726	,,	37	R	Feb. 22	1851	9000	Ord.	39	—
							1907. Feb. 6	2624	3000	,,	66	—

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop. R†—Reaction and died.

IMMUNISATION OF HORSES WITH ORDINARY VIRUS.—(*cont.*

Test.						Test.					
Date of Injection.	Virus.				Result.	Date of Injection.	Virus.				Result.
	No.	Qu.	Orig.	Gen.			No.	Qu.	Orig.	Gen.	
1906.		c.c.				1906.		c.c.			
Jan. 20	1588	9000	Ord.	61	—	April 13	1911	5500	Elder	3	—
						April 30	1893	3500	Turnbull	5	—
Mar. 7	1862	9000	,,	60	—	June 22	2027	9000	Tzn.	3	RD†
1905.											
Dec. 31	1504	3000	..	40	—	April 26	1857	9000	Turnbull	4	—
1906.											
Jan. 17	1542	5500	,,	61	—						
1907.											
Feb. 12	2632	4000	..	67	—						
Feb. 14	2636	2000	..	68	—						
1906.											
Mar. 24	1892	6000	Turnbull	2	—						
Mar. 28	1645	3000	,,	3	—						
Mar. 20	1398	3000	Altenroxel	1	?	June 22	2032	6000	Tzn.	3	RD
Mar. 24	1892	6000	Turnbull	2	?	June 23	2027	3000	..	3	RD
May 23	1960	9000	..	8	?	July 18	2058	40	,,	6	R
1907.											
Feb. 7	2625	6000	Ord.	66	—						
1906.						1907.					
June 3	2001	8500	Tzn.	2	R	Feb. 15	2636	3000	Ord.	68	—
						Feb. 20	2637	3000	..	69	—
						1906.					
April 26	1857	9000	Turnbull	4	—	July 12	2060	6000	Bul.	2	—
						July 20	1959	1500	..	3	—
May 23	1960	9000	,,	8	—	July 12	2059	6000	Tzn.	6	R
						Aug. 24	2090	3000	,,	9	R

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop.
RD†—Reaction with Dikkop and died.

IMMUNISATION OF HORSES WITH ORDINARY VIRUS.—(*cont.*)

Horse No.	Immunisation.						Test.					
	Date of Injection.	Serum.	Virus.			Result.	Date of Injection.	Virus.				Result.
		Orig.	No.	Orig.	Gen.			No.	Qu.	Orig.	Gen.	
1654	1906. Jan. 31	Ord.	726	Ord.	37	RD	1906. Mar. 8	1862	c.c. 8500	Ord.	60	—
							Aug. 24	2093	1500	Bul.	6	—
							Aug. 29	2106	7500	,,	7	—
1775	,,	,,	726	,,	37	R	Mar. 29	1645	9000	Turnbull	3	—
1781	,,	,,	726	,,	37	RD	Mar. 24	1892	3000	,,	2	—
							Mar. 28	1645	6000	,,	3	—
1830	May 11	,,	1863	,,	61	R	June 8	2006	3000	Elder	3	—
							June 14	2038	6000	,,	4	—
1904	July 12	,,	1863	,,	61	RD	Aug. 2	1869	1	Tzn.	1	R
1943	Oct. 19	,,	1938	,,	62	?	Nov. 22	1869	2	,,	1	—
1958	April 25	,,	1863	,,	61	R	June 29	2040	9000	,,	4	RD
							Dec. 7	1954	2	Bul.	1	—
1972	June 18	,,	1863	,,	61	R	July 20	2014	9000	Tzn.	7	R
2003	June 1	,,	1863	,,	61	R	June 27	2028	9000	,,	4	RD
							Dec. 6	2409	9000	OTB LPW	1	—
2004	June 2	,,	1863	,,	61	R	June 27	2028	9000	Tzn.	4	R
2011	May 29	,,	1863	,,	61	R	June 29	2040	9000	,,	4	R
2016	June 30	,,	1863	,,	61	RD	July 19	2060	20	Bul.	2	RD†
2019	,,	,,	1863	,,	61	R	,,	2058	20	Tzn.	6	—
2021	July 12	.	1863	,,	61	RD	Aug. 2	1964	1	Bul.	2	RD†
2022	,,	,,	1863	,,	61	RD	,,	1869	1	Tzn.	1	—
							Sept. 26	2225	9000	Bul.	3	RD†
2077	,,	,,	1863	,,	61	RD	Aug. 2	1964	1	,,	2	—
2307	Oct. 6	T-B	1938	,,	62	R	Nov. 22	1959	2	,,	3	-
2390	Nov. 7	O-T-B	1938	,,	62	RD	Dec. 7	2287	2	Ord.	38	—
2419	Dec. 6	OTB	2407	,,	38	RD	1907. Jan. 10	2199	10	Tzn.	12	R

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop.
RD†—Reaction with Dikkop and died.

IMMUNISATION OF HORSES WITH ORDINARY VIRUS.—(*cont.*)

Test.						Test.					
Date of Injection.	Virus.				Result.	Date of Injection.	Virus.				Result.
	No.	Qu.	Orig.	Gen.			No.	Qu.	Orig.	Gen.	
1906.		c.c.				1906.		c.c.			
June 2	1990	9000	Elder	2	—	July 21	2060	10	Bul.	2	—
						1907.					
Nov. 20	2402	9000	OTB	4	—	Jan. 25	2599	3000	Spont. C.	1	R†
June 22	2029	6000	Elder	4	R						
June 28	2028	3000	Tzn.	4	R						
June 22	2029	3000	Elder	4	R						
June 23	2027	3000	Tzn.	4	R						
1907.											
Feb. 12	2632	3000	Ord.	67	—						
1906.											
Dec. 6	2409	9000	OTB LPW	1	—						
						1906.					
Aug. 31	2090	5000	Tzn.	9	—	Nov. 21	1938	2	Ord.	62	—
,,	2152	3500	,,	2	—						
1907.											
Feb. 14	2636	6000	Ord.	68	—						
1906.											
Nov. 21	1938	2	,,	62	—	Dec. 7	1954	2	Bul.	1	—
Sept. 5	2151	9000	Tzn.	10	—	Nov. 21	1938	2	Ord.	62	—
Nov. 21	1938	2	Ord.	62	—	Dec. 7	1954	2	Bul.	1	R
Aug. 17	1964	1	Bul.	2	—	Aug. 29	2111	8500	,,	7	—
Aug. 17	1964	1	,,	2	—	Sept. 1	1964	5	,,	2	—
,,	1964	10	,,	2	RD†						
Dec. 7	1954	2	,,	1	—						
1907.											
Jan. 18	2552	9000	Ord.	64	—						

R—Reaction. R†—Reaction and died. RD†—Reaction with Dikkop and died.

Analysis from Preceding Tables.

104 horses, immunised with Ordinary virus were hyperimmunised and tested as follows :—

16 horses, once hyperimmunised with O : No reactions.

10 ,, twice ,, ,, ,,

5 ,, three times hyperimmunised with O : No reactions.

1 horse, 1st hyperimmunised with O, 2nd with hyperimmunised Tzaneen, and 3rd hyperimmunised with O : A reaction with 2nd test.

1 horse, twice hyperimmunised with O and then hyperimmunised with Tzaneen, had reaction, dikkop, and died.

3 horses, four times hyperimmunised with O, 5th hyperimmunised with Tzaneen, and 6th hyperimmunised with O : 2 had reaction and dikkop when hyperimmunised with Tzaneen.

1 horse, 1st hyperimmunised with O, 2nd and 3rd time tested with Tzaneen, 4th and 5th tested with Bulawayo, 6th hyperimmunised with Bulawayo, and 7th hyperimmunised with OTB, had a doubtful reaction with 6th test.

1 horse, five times hyperimmunised with O, 6th tested with Tzaneen, 7th hyperimmunised with Tzaneen, and 8th hyperimmunised with O : No reaction.

1 horse was twice tested with O and had no reaction.

1 horse, four times hyperimmunised with O, 5th hyperimmunised with Bulawayo, 6th hyperimmunised with OTBLPW, and 7th hyperimmunised with Spontaneous Case + OTBLPW : Reaction and dikkop with 5th test.

1 horse, four times hyperimmunised with O : No reaction. Tested with Bulawayo gave reaction and dikkop.

1 horse, five times hyperimmunised with O, 6th tested with Bulawayo, 7th hyperimmunised with Bulawayo, and 8th hyperimmunised with OTBLPW : No reaction.

2 horses, 1st hyperimmunised with O, 2nd hyperimmunised with Spont. C., 3rd hyperimmunised with Tzaneen, 4th hyperimmunised with O : 1 had doubtful reaction with 2nd test and reaction and dikkop with 3rd test ; 1 a reaction with 3rd test.

1 horse, 1st hyperimmunised with O, 2nd hyperimmunised with Spont. C., 3rd tested with Tzaneen, 4th hyperimmunised with Tzaneen, and 5th hyperimmunised with O : Had a doubtful reaction with 2nd test ; reaction with 3rd test and also with 4th test.

3 horses, twice hyperimmunised with O and then hyperimmunised with Spont. C. : No reactions.

1 horse, twice hyperimmunised with O 3rd, hyperimmunised with Spont C., and 4th hyperimmunised with O : Reaction when hyperimmunised with Spont. C.

1 horse, twice hyperimmunised with O, 3rd hyperimmunised with Spont. C., 4th hyperimmunised with O, 5th and 6th tested with Bulawayo, and 7th hyperimmunised with OTBLPW : Reaction with last test.

5 horses, twice hyperimmunised with O, 3rd hyperimmunised with Spont. C., and 4th hyperimmunised with Tzaneen: 2 had reactions when hyperimmunised with Tzaneen, and 2 others had reactions, dikkop, and died.

2 horses, twice hyperimmunised with O, 3rd hyperimmunised with Spont. C., 4th hyperimmunised with Tzaneen, and 5th hyperimmunised with O: No reaction.

1 horse, twice hyperimmunised with O, 3rd and 4th hyperimmunised with Spont. C., and 5th tested with Tzaneen: Reaction, dikkop, and died on last test.

1 horse, twice hyperimmunised with O, 3rd hyperimmunised with Spont. C., and 4th $\left\{\begin{matrix}\text{Tzn.}\\\text{Spont.}\end{matrix}\right\}$: Had a reaction with Spont. C. reaction and dikkop with hyperimmunised $\left\{\begin{matrix}\text{Tzn.}\\\text{Spont.}\end{matrix}\right\}$ and later on a relapse of dikkop spontaneously contracted at Onderstepoort, Pretoria District.

2 horses, thrice hyperimmunised with O, and 4th hyperimmunised with Spont. C.: No reactions.

3 horses, thrice hyperimmunised with O, 4th hyperimmunised with Spont. C., and 5th hyperimmunised with O: 1 had a doubtful reaction when hyperimmunised with Spont. C.

1 horse, thrice hyperimmunised with O, 4th hyperimmunised with Spont. C., 5th hyperimmunised with O, 6th and 7th tested with Bulawayo, 8th hyperimmunised with Bulawayo, and 9th hyperimmunised with OTBLPW: No reaction.

1 horse, thrice hyperimmunised with O, 4th hyperimmunised with $\left\{\begin{matrix}\text{Tzn.}\\\text{Spont.}\end{matrix}\right\}$ and 5th hyperimmunised with O: Had a doubtful reaction with 1st hyperimmunised O, and reaction and dikkop with hyperimmunised $\left\{\begin{matrix}\text{Tzn.}\\\text{Spont.}\end{matrix}\right\}$

2 horses, thrice hyperimmunised with O, 4th hyperimmunised with Spont. C., and 5th hyperimmunised with Tzaneen: Reactions, dikkop, and died.

5 horses, thrice hyperimmunised with O, 4th hyperimmunised with Spont. C., 5th hyperimmunised with Tzaneen, and 6th hyperimmunised with O: 1 had a doubtful reaction with 1st test and a reaction when hyperimmunised with Tzaneen; 1 had reaction and dikkop when hyperimmunised with Tzaneen.

1 horse, thrice hyperimmunised with O, 4th hyperimmunised with Spont. C., 5th and 6th tested with Tzaneen, 7th and 8th tested with Bulawayo, and 9th hyperimmunised with OTB: No reaction.

2 horses, four times hyperimmunised with O, 5th hyperimmunised with $\left\{\begin{matrix}\text{Spont.}\\\text{Tzn.}\end{matrix}\right\}$ and 6th hyperimmunised with O: 1 had a reaction and dikkop with the 6th test (O).

1 horse, four times hyperimmunised with O, 5th hyperimmunised with Spont. C., 6th tested with Tzaneen, 7th and 8th tested with Bulawayo, 9th hyperimmunised with Bulawayo, and 10th hyperimmunised with OTBLPW : No reaction.

1 horse, first hyperimmunised with O, 2nd hyperimmunised with Spont. C., 3rd hyperimmunised with Bulawayo, and 4th hyperimmunised with OTB : No reaction.

1 horse, first hyperimmunised with O, 2nd hyperimmunised with Spont. C., 3rd tested with Bulawayo, 4th hyperimmunised with Bulawayo, 5th hyperimmunised with OTB, and 6th hyperimmunised with Spont. C. Reaction and died.

1 horse, thrice hyperimmunised with O, 4th hyperimmunised with Spont. C., 5th hyperimmunised with Bulawayo, and 6th hyperimmunised with OTB : No reaction.

1 horse, twice hyperimmunised with O, 3rd test hyperimmunised with Spont., 4th test with Bulawayo, and 5th hyperimmunised with OTB : Reaction with 4th test.

1 horse, thrice hyperimmunised with O, 4th hyperimmunised with Spont. C., 5th and 6th tested with Bulawayo, 7th hyperimmunised with Bulawayo, and 8th hyperimmunised with OTB : No reaction.

1 horse, thrice hyperimmunised with O, 4th hyperimmunised with Spont. C., 5th tested with Bulawayo, 6th hyperimmunised with Bulawayo, and 7th hyperimmunised with OTB : Had a doubtful reaction with 3rd hyperimmunised O. and reaction and dikkop with the Bulawayo test.

1 horse, four times hyperimmunised with O, 5th hyperimmunised with Spont. C., and tested 6th with Bulawayo : Reaction with Bulawayo test.

1 horse was hyperimmunised with Tzaneen and had a reaction.

1 horse, 1st and 2nd hyperimmunised with Tzaneen, 3rd tested with O, and 4th hyperimmunised with OTBLPW had reaction and dikkop with 1st hyperimmunised Tzaneen.

1 horse, 1st and 2nd hyperimmunised with Tzaneen, 3rd tested with O, 4th tested with Bulawayo, and 5th hyperimmunised with O had reaction and dikkop with the 1st hyperimmunised Tzaneen.

2 horses, 1st hyperimmunised with Tzaneen, 2nd tested with O, and 3rd tested with Bulawayo : 1 had a reaction when hyperimmunised with Tzaneen ; 1 had reactions when hyperimmunised with Tzaneen and tested with Bulawayo.

3 horses were tested with Tzaneen : 2 had reactions and 1 had a reaction and died.

1 horse was 1st tested with Tzaneen and 2nd with OTBLPW : No reaction.

2 horses, first tested with Tzaneen, 2nd tested with Bulawayo, and 3rd hyperimmunised with Bulawayo : 1 had reaction, dikkop, and died ; 1 had no reaction.

1 horses were tested with Bulawayo: 1 had reaction and dikkop, and 3 had reactions, dikkop, and died.

2 horses were twice tested with Bulawayo: 1 had a reaction, dikkop, and died on 2nd test.

1 horse, 1st hyperimmunised with Spont. C., and 2nd hyperimmunised with O: No reaction.

2 horses, hyperimmunised with Spont. C., and 2nd hyperimmunised with { Tzn. / Spont. } both had reactions with 2nd hyperimmunisation.

2 horses, immunised with mule virus 1489, viz., Ordinary virus passed through donkey 1599:—

1 horse was tested with Tzaneen: Reaction and died.

1 horse tested with Bulawayo: Reaction and died.

1 horse immunised with mule virus 1487 viz., Ordinary virus passed through goats 375, 378, and 381 was first hyperimmunised with O, 2nd hyperimmunised with Spont. C., and 3rd hyperimmunised with Tzaneen: Reaction and died.

4 horses immunised with virus X (mixture of blood from goats 375, 378, and 381, injected with Ordinary virus):—

1 horse was hyperimmunised with O: No reaction.

1 horse, 1st hyperimmunised with O, 2nd hyperimmunised with Spont. C., and 3rd hyperimmunised with Tzaneen: Reaction and dikkop when hyperimmunised with Tzaneen.

1 horse 1st hyperimmunised with O, and 2nd hyperimmunised with { Tzn. / Ord. }: No reaction.

1 horse, 1st hyperimmunised with O, 2nd hyperimmunised with Spont. C., 3rd tested with Tzaneen, 4th tested with Bulawayo, and 5th hyperimmunised with Bulawayo: A doubtful reaction with the 3rd test and a distinct reaction with the 4th test.

Summary of Results of Tests on Horses Previously Immunised with Ordinary Virus.

No. of Horses.	Tested with.	Result.			
		Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Died.
88	Ordinary Virus	—	1	3	—
40	Tzaneen	12	6	—	7
26	Bulawayo	3	4	1	5
10	OTB	—	—	—	—
5	OTBLPW	1	—	—	—
43	Spontaneous Case	2	—	3	1
5	Tzaneen and Spont. C. ..	2	2	—	—
1	Ord. and Spont. C.	—	—	—	—
1	OTBLPW and Spont. C. ..	—	—	—	—

Immunisation of Horses with Ordinary Virus (Passed through Donkeys).

Horse No.	Immunisation. Date of Injection.	Serum. Orig.	Virus. No.	Virus. Orig.	Virus. Gen.	Result.	Test. Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.
							1906.					
1653	Jan. 26	Ord.	1506	Ord.	39	RD	Feb. 24	1855	6000	Ord.	40	—
							Feb. 26	1854	3000	,,	64	—
1669	,,	,,	1506	,,	39	R	Feb. 22	1853	6000	,,	64	—
							March 6	1862	3000	,,	60	—
1674	,,	,,	1506	,,	39	R	Aug. 15	2089	9000	,,	63	—
1859	July 18	,,	1506	,,	39	R	,,	2089	6000	,,	63	—
1988	July 12	,,	1506	,,	39	RD	Aug. 17	1964	10	Bul.	2	—
2010	June 28	,,	1506	,,	39	R	July 19	2060	20	,,	2	R†
2012	June 29	,,	1506	,,	39	R	July 20	2014	9000	Tzn.	7	RD†
2037	July 18	,,	1506	,,	39	R	Aug. 17	1964	1	Bul.	2	—
							Oct. 29	2357	5000	OTB	2	—
							Dec. 6	2418	3000	OTB	5	—
2041	July 12	,,	1506	,,	39	R	Aug. 2	1869	1	Tzn.	1	—
2043	July 18	,,	1506	,,	39	R	Aug. 17	1964	1	Bul.	2	—
1882	June 28	,,	1489	,,	61	RD	July 19	2058	20	Tzn.	6	R†
1971	,,	,,	1489	,,	61	R	Aug. 17	1964	1	Bul.	2	R†
	1905.						1906.					
1507	Dec. 19	,,	1487	,,	1	R	Jan. 27	1584	6000	Ord.	62	—
							Jan. 29	1527	3000	,,	40	—
1505	,,	,,	X	,,	1	R	Jan. 27	1584	9000	,,	62	—
1578	,,	,,	X	,,	1	R	,,	1584	9000	,,	62	—
1580	,,	,,	X	,,	1	RD	,,	1541	5500	,,	62	—
							Jan. 29	1527	3500	,,	40	—
1582	,,	,,	X	,,	1	R	Jan. 21	1588	3000	,,	61	—
							Jan. 27	1541	6000	,,	62	—
							Aug. 17	1964	1	Bul.	2	R

R—Reaction. RD—Reaction with Dikkop. R†—Reaction and Died.
RD†—Reaction with Dikkop and Died.

Analysis from Preceding Table.

10 horses, immunised with horse virus 1506, viz., Ordinary virus passed through donkeys 1427, 1429, 1430, 1431 :—

1 horse, hyperimmunised with Ordinary : No reaction.
1 horse, twice hyperimmunised with Ordinary : No reaction.
1 horse, 1st hyperimmunised with O, 2nd hyperimmunised with Tzaneen, and 3rd hyperimmunised with O : Reaction with Tzaneen.
1 horse, 1st hyperimmunised with O, 2nd hyperimmunised with Spont. C, and 3rd tested with Bulawayo : Reaction, dikkop, and died.
1 horse, hyperimmunised with Tzaneen : Reaction, dikkop, and died.
1 horse, 1st tested with Tzaneen, and 2nd test with Bulawayo. Reaction, dikkop, and died.
1 horse, tested with Bulawayo : Reaction and died.
1 horse, 1st test with Bulawayo, 2nd hyperimmunised with Bulawayo : No reaction.
1 horse, 1st and 2nd test with Bulawayo, and 3rd hyperimmunised with Bulawayo : Reaction and dikkop when hyperimmunised with Bulawayo.
1 horse, 1st and 2nd test with Bulawayo, 3rd hyperimmunised with Bulawayo, and 4th hyperimmunised with OTB : No reaction.

IMMUNISATION OF HORSES WITH ORDINARY VIRUS (PASSED THROUGH DONKEYS).

Test.						Test.					
Date of Injection.	Virus.				Result.	Date of Injection.	Virus.				Result.
	No.	Qu.	Orig.	Gen.			No.	Qu.	Orig.	Gen.	
1906.						1906.					
May 23	1974	6000	Turnbull	8	—	Aug. 3	1964	1	Bul.	2	RD†
May 31	1990	3000	Elder	2	—						
						1907.					
June 2	2001	9000	Tzn.	2	R	Feb. 6	2625	3000	Ord.	66	—
						,,	2626	3000	,,	66	—
1907.											
Feb. 14	2636	3000	Ord.	68	—						
Feb. 19	2637	3000	,,	69	—						
1906.											
Aug. 28	2109	9000	Bul.	7	—						
1906.						1906.					
Sept. 1	1964	5	Bul.	2	—	Oct. 10	2150	8500	Bul.	12	—
Aug. 17	1964	1	,,	2	RD†						
Sept. 1	1964	5	,,	2	—	Sept. 26	2225	9000	Bul.	3	RD
April 26	1857	8000	Turnbull	4	—	July 13	2059	9000	Tzn.	6	R†
May 1	1893	9000	Turnbull	5	—	June 28	2028	9000	Tzn.	4	RD
June 2	2001	3000	Tzn.	2	—						
June 6	2006	6000	Ord.	3	—						
May 1	1893	9000	Turnbull	5	—	Aug. 3	1869	1	,,	1	?
Aug. 29	2109	9000	Bul.	7	—						

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop.
RD†—Reaction with Dikkop and Died.

SUMMARY OF RESULTS OF TESTS ON HORSES PREVIOUSLY IMMUNISED WITH HORSE VIRUS 1506.

(Viz., Ordinary virus passed through Donkeys 1427, 1429, 1430, 1431.)

No. of Horses.	Tested with.	Result.			
		Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Died.
4	Ordinary Virus	—	—	—	—
3	Tzaneen	1	—	—	1
6	Bulawayo	—	1	—	3
1	OTB	—	—	—	—
1	Spontaneous Case	—	—	—	—

Immunisation of Horses with Ordinary Virus.

Passed through a Donkey.

Horse No.	Immunisation. Date of Injection.	Serum. Orig.	Virus. No.	Virus. Orig.	Virus. Gen.	Result.	Test. Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.
	1905.						1906.		c.c.			
1399	Dec. 19	Ord.	1427	Ord.	38	R	Jan. 26	1526	9000	Ord.	62	R†
	1906.											
1406	Jan. 31	,,	1427	,,	38	R	Mar. 15	1863	9000	,,	61	R
1656	Jan. 26	,,	1427	,,	38	R	Mar. 8	1788	2	Dale	1	RD
1659	,,	,,	1427	,,	38	RD	Feb. 28	1854	9000	Ord.	64	—
							Aug. 2	1964	20	Bul.	2	—
							Dec. 4	2418	3000	OTB	5	—
1660	,,	,,	1427	,,	38	R	Feb. 24	1855	5500	Ord.	40	?
							Feb. 26	1853	3000	,,	64	?
1661	,,	,,	1427	,,	38	RD	Feb. 27	1853	6000	,,	64	—
							Mar. 6	1862	3000	,,	60	—
							Aug. 10	2082	9000	Tzn.	8	—
1665	Jan. 31	,,	1427	,,	38	R	Mar. 8	1862	9000	Ord.	60	—
							1907.					
							Feb. 15	2636	3000	,,	68	—
							Feb. 19	2637	3000	,,	69	—
							1906.					
1672	Feb. 12	,,	1427	,,	38	RD	April 6	1880	6000	Turn-bull	3	—
							April 8	1891	3000	Elder	2	—
1866	Mar. 24	,,	1427	,,	38	R	May 3	1893	9000	Turn-bull	5	R
1973	May 25	,,	1427	,,	38	RD	June 28	2026	9000	Ord.	5	R
							Oct. 5	2199	9000	Tzn.	12	—
2023	June 8	,,	1427	,,	38	R	July 19	2060	20	Bul.	2	R†

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop. R†—Reaction and Died.

Immunisation of Horses with Ordinary Virus, Passed through a Donkey.

Test.						Test.					
Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.	Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.
		c.c.						c.c.			
1906.						1907.					
June 2	2001	9000	Tzn.	2	R	Feb. 14	2635	3000	Ord.	68	—
						1906.					
June 6	2006	9000	Ord.	3	—	Aug. 15	2089	5000	,,	63	?
Mar. 23	1974	6000	Turn-bull	8	—	July 21	2060	10	Bul.	2	—
May 31	1990	3000	Elder	2	—						
Aug. 15	2086	2000	Bul.	5	—	Nov. 22	2403	9000	OTB	4	—
Aug. 24	2093	5000	,,	6	—						
						1907.					
May 23	1960	6000	Turn-bull	8	?	Feb. 12	2630	3000	Ord.	67	—
May 31	1990	3000	Elder	2	?	Feb. 20	2637	3000	,,	69	—
						1906.					
June 1	1960	2500	Turn-bull	8	—	July 21	2058	10	Tzn.	6	R
,,	1990	6500	Elder	2	—						
1907.											
Feb. 12	2634	3000	Ord.	67	—						
Feb. 14	2635	3000	,,	68	—						
1906.											
June 1	1990	6000	Elder	2	R	Sept. 6	2151	9000	Tzn.	10	—
,,	2001	3000	Tzn.	2	R						
						1907.					
June 23	2027	8500	Tzn.	3	RD	Feb. 14	2635	6000	Ord.	68	—
July 12	2060	9000	Bul.	2	R†						
						1906.					
Aug. 3	1964	1	,,	2	RD	Aug. 24	2093	3000	Bul.	6	—
						Aug. 28	2106	6000	,,	7	—

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop. R†—Reaction and Died.

Analysis from Preceding Table.

11 horses, immunised with donkey virus 1427 (donkey 1427 was injected with Ord. virus 726).

1 horse was hyperimmunised with O: Reaction and died.

1 horse, 1st hyperimmunised with O, 2nd hyperimmunised with Tzaneen, 3rd hyperimmunised with Ord.: Reaction with 1st hyperimmunisation O, and also reaction when hyperimmunised with Tzaneen.

1 horse, 1st hyperimmunised with O, 2nd hyperimmunised with Spont. C., and 3rd hyperimmunised with O: Doubtful reactions with the 1st and 2nd hyperimmunisations.

1 horse, hyperimmunised with O, 2nd hyperimmunised with Spont. C., 3rd and 4th tested with Bulawayo, 5th hyperimmunised with Bulawayo, 6th and 7th hyperimmunised with OTB: No reaction.

1 horse, 1st hyperimmunised with O, 2nd hyperimmunised with Spont. C., 3rd tested with Tzaneen, 4th hyperimmunised with Tzaneen, and 5th hyperimmunised with O: Reaction with the 3rd test.

1 horse, 1st hyperimmunised with O, 2nd hyperimmunised with {Tzn. / Spont.}, 3rd hyperimmunised with Tzaneen, and 4th hyperimmunised with O: Reaction when hyperimmunised with {Tzn. / Spont.}

1 horse, 1st hyperimmunised with O, 2nd tested with Bulawayo, 3rd hyperimmunised with Bulawayo, and 4th hyperimmunised with Tzaneen: Reaction when hyperimmunised with O and reaction and dikkop with the Bulawayo test.

1 horse was tested with Bulawayo: Reaction and died.

1 horse, 1st test with Spont. C., 2nd and 3rd hyperimmunised with O: Reaction and dikkop with test Spont. C., and a doubtful reaction with 3rd hyperimmunisation.

1 horse, 1st hyperimmunised with Spont. C., 2nd hyperimmunised with Tzaneen, and 3rd hyperimmunised with O: Reaction and dikkop when hyperimmunised with Tzaneen.

1 horse, 1st hyperimmunised with Spont. C., 2nd hyperimmunised with Bulawayo: Reaction with 1st hyperimmunisation, and reaction and died 2nd hyperimmunisation.

Summary of Results of Tests on Horses Previously Immunised with Donkey Virus 1427 (Ord. Virus).

No. of Horses.	Tested with.	Result.			
		Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Died.
11	Ordinary Virus	2	—	2	1
5	Tzaneen	2	1	—	—
5	Bulawayo	—	1	—	2
1	OTB	—	—	—	—
6	Spontaneous Case	1	1	1	—
1	Tzaneen and Spont. C. ..	1	—	—	—

Immunisation of Horses with Tzaneen Virus.

Horse No.	Immunisation. Date of Injection.	Serum. Orig.	Virus. No.	Virus. Orig.	Virus. Gen.	Result.	Test. Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.
	1906.						1906.		c.c.			
1909	July 12	Ord.	1869	Tzn.	1	RD	Aug. 2	1863	10	Ord.	61	—
1915	Aug. 2	Tzn.	1869	,,	1	RD	Oct. 9	2148	8500	Tzn.	13	R†
1944	June 14	Ord.	1869	,,	1	R	July 19	2060	20	Bul.	2	—
							Nov. 21	1938	2	Ord.	62	—
1989	June 26	,,	1869	,,	1	R	July 19	2060	20	Bul.	2	R
2008	July 12	,,	1869	,,	1	RD	Aug. 2	1863	10	Ord.	61	—
2079	July 12	,,	1869	,,	1	RD	,,	1964	10	Bul.	2	R
2081	,,	,,	1869	,,	1	R	,,	1964	10	,,	2	RD
							Dec. 6–11	2406	9000	OTB	1	—
								2409		LPW	1	
								2416			2	
2110	Sept. 27	Bul.	1869	,,	1	R						
	Nov. 8	,,	2199	,,	12	RD	Dec. 7	2267	2	Tzn.	3	—
2128	Aug. 2	Tzn.	1869	,,	1	R						
	Oct. 19	Bul.	2199	,,	12	R	Nov. 22	726	2	Ord.	37	?
2135	Aug. 2	Tzn.	1869	,,	1	RD	Sept. 21	2171	9000	Tzn.	11	R
2140	Nov. 8	OB* (H&D)	2199	,,	12	R	Dec. 7	2267	2	,,	3	R
2278	Oct. 19	Bul.	2199	,,	12	RD	Nov. 22	1938	2	Ord.	62	R
2310	Oct. 6	,,	1869	,,	1	RD	,,	1938	2	,,	62	R
							1907.					
2098	Oct. 11	Bul.	2169	,,	3	R	Feb. 8	2407	2	Ord.	38	—
							1906.					
2198	Sept. 27	,,	2169	,,	3	R	Oct. 24	2153	3000	OTB	1	R†
2238	Oct. 12	,,	2169	,,	3	R	Nov. 5	2208	5	Bul.	2	—
							1907.					
2244	Oct. 11	,,	2169	,,	3	RD	Feb. 8	2407	2	Ord.	38	R†
							1906.					
2205	Sept. 27	Bul.	2267	,,	3	R	Oct. 24	2153	6000	OTB	1	R†
2349	Oct. 22	Ord.	2267	,,	3	R	Nov. 22	2199	2	Tzn.	12	—
2350	,,	Bul.	2267	,,	3	R	,,	2199	2	,,	12	—
2351	,,	,,	2267	,,	3	R	,,	2199	2	,,	12	R†
2353	,,	,,	2267	,,	3	?	,,	2199	2	,,	12	RD†
2354	,,	,,	2267	,,	3	R	,,	2199	2	,,	12	R
2355	,,	,,	2267	,,	3	?	,,	2199	2	,,	12	RD†

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop.
R†—Reaction and died. RD†—Reaction with Dikkop and died.
*Note.—Mixture of Ordinary and Bulawayo Serum of Horses and Donkeys.

Immunisation of Horses with Tzaneen Virus.

Test.						Test.					
Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.	Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.
1906.		c.c.				1906.		c.c.			
Aug. 17	1964	1	Bul.	2	RD						
Aug. 11	2083	9000	Bul.	4	—	Oct. 9	2148	8000	Tzn.	13	R
Dec. 6	2406	9000	OTB LPW	1	—						
Aug. 10	2083	9000	Bul.	4	—						
Aug. 17	1964	1	,,	2	R	Aug. 28	2109	2000	Bul.	7	—
						,,	2111	3000	,,	7	—
						Sept. 3	2149	3000	,,	8	—
Aug. 24	2093	9000	,,	6	—						
Aug. 19	2093	9000	,,	6	—	Nov. 21	1938	2	Ord.	62	R
1907.						1907.					
Jan. 10	2496	10	Ord.	63	—	Feb. 23	2640	9000	OTB LPW	2	R
1906.						1907.					
Dec. 7	1954	2	Bul.	1	—	Jan. 19	2552	8500	Ord.	64	R†
Nov. 21	1938	2	Ord.	62	R†						
1907.											
Feb. 8	2407	2	,,	38	R						
						1907.					
Dec. 13	1954	2	Bul.	1	—	Jan. 10	2496	6000	Ord.	63	—
						Jan. 28	2553	3000	,,	65	—
,,	1954	2	,,	1	—	Jan. 10	2496	6000	,,	63	—
						Jan. 28	2553	3000	,,	65	—
1907.											
Feb. 8	2407	2	Ord.	38	R†						
1906.	1938		O	62							
Dec. 13	2199	2	T	12	—						
	2298		B	11							
	1938		O	62							
,,	2199	2	T	12	—						
	2298		B	11							
	1938		O	62							
Dec. 13	2199	2	T	12	R†						
	2298		B	11							

R = Reaction. RD—Reaction with Dikkop. R†—Reaction and died.

Analysis from Preceding Table.

13 horses, immunised with Tzaneen virus.

1 horse, 1st test with O, and 2nd test with Bulawayo: Reaction and dikkop with the 2nd test.

1 horse, 1st test with O, 2nd tested with Bulawayo, and 3rd hyperimmunised with Bulawayo: Reaction with the 2nd test.

3 horses, 1st tested with O, 2nd tested with Bulawayo, and 3rd hyperimmunised with O: 2 had reactions with the 1st test: 1 a doubtful reaction with the 1st test, and reaction and died when hyperimmunised with O.

1 horse was hyperimmunised with Tzaneen: Reaction and died.

1 horse, hyperimmunised with Tzaneen and 2nd tested with O: Reaction when hyperimmunised with Tzaneen, and reaction and died with the 2nd test.

1 horse, 1st tested with Tzaneen and 2nd tested with O.: Reaction with both tests.

1 horse, 1st test with Tzaneen, 2nd tested with O, and 3rd hyperimmunised with OTBLPW: Reaction when hyperimmunised with OTBLPW.

2 horses, 1st tested with Bulawayo, 2nd hyperimmunised with Bulawayo: Both had reactions with the 1st test.

1 horse, 1st tested with Bulawayo, 2nd hyperimmunised with Bulawayo, 3rd tested with O, and 4th hyperimmunised with OTBLPW: Reaction and dikkop with the 1st test, and a reaction with the 3rd test.

1 horse, 1st tested with Bulawayo, 2nd hyperimmunised with Bulawayo, 3rd hyperimmunised with Tzaneen, 4th tested with O, and 5th hyperimmunised with OTBLPW: Reaction with 3rd test.

4 horses, immunised with horse virus 2169, viz., Tzaneen virus passed through donkey 1773.

2 horses were tested with O : 1 had a reaction and died.

1 horse, 1st tested with Bulawayo, and 2nd tested with O : Reaction and died with the 2nd test.

1 horse was hyperimmunised with OTB : Reaction and died.

7 horses, immunised with mule virus 2267 (viz., Tzaneen virus passed through donkey 1773).

3 horses were tested with Tzaneen : 1 had a reaction and died ; 2 had reactions, dikkop, and died.

3 horses, 1st tested with Tzaneen, and 2nd tested with $\left\{\begin{matrix} O \\ T \\ B \end{matrix}\right\}$: 1 had a reaction with the 1st test and reaction and died with the 2nd test.

1 horse was hyperimmunised with OTB : Reaction and died.

Summary of Results of Tests on Horses Previously Immunised with Tzaneen Virus.

No. of Horses.	Tested with.	Result.			
		Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Died.
10	Ordinary Virus	4	—	4	2
5	Tzaneen	3	—	—	1
9	Bulawayo	3	2	—	—
3	OTBLPW	1	—	—	—

Immunisation of Horses with Bulawayo Virus.

Horse No.	Immunisation. Date of Injection.	Serum. Orig.	Virus. No.	Virus. Orig.	Virus. Gen.	Result.	Test. Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.
	1906.						1906.		c.c.			
1946	Aug. 16	Bul.	1964	Bul.	2	R	Sept. 4	2149	6000	Bul.	8	—
							Sept. 12	2172	3000	,,	9	—
1953	,,	B-T*	1964	,,	2	R	,,	2172	9000	,,	9	R
1977	,,	Bul.	1964	,,	2	R	,,	2172	3000	,,	9	—
							Sept. 18	2168	6000	,,	10	—
2124	Sept. 20	,,	1964	,,	2	RD	Oct. 21	2167	9000	Tzn.	5	—
2125	Sept. 28	,,	1964	,,	2	RD	Oct. 17	1938	10	Ord.	62	R
2132	Sept. 20	Tzn.	1964	,,	2	RD	Oct. 21	2167	9000	Tzn.	5	R†
2176	Aug. 16	B-T*	1964	,,	2	R	Sept. 17	2172	6000	Bul.	9	—
							,,	2168	3000	,,	10	—
2272	Sept. 20	Bul.	1964	,,	2	RD	Oct. 22	2153	6000	OTB	1	R†
							1906.					
2047	Sept. 27	,,	2201	,,	3	RD	Oct. 17	1938	10	Ord.	62	R
2239	Oct. 11	,,	2201	,,	3	RD	Nov. 5	2208	5	Bul.	2	—
	1907.						1907.					
2425	Jan. 30	OTB	2201	,,	3	RD	Jan. 10	2496	10	Ord.	63	R

R—Reaction. RD—Reaction with Dikkop. R†—Reaction and Died.
* A mixture of Bulawayo and Tzaneen Serum.

Analysis from Preceding Table.

8 horses, immunised with Bulawayo virus, were tested as follows :—

4 horses were hyperimmunised with Bulawayo : 1 had a reaction.

1 horse was hyperimmunised with Tzaneen : Reaction and died.

1 horse, 1st hyperimmunised with Tzaneen, 2nd tested with O, and 3rd hyperimmunised with OTBLPW : Reaction with 2nd test.

1 horse, hyperimmunised with OTB : Reaction and died.

1 horse, 1st tested with O, and 2nd hyperimmunised with OTB : Distinct reaction with 1st test and doubtful reaction with 2nd test.

3 horses, immunised with horse virus 2201 (viz., Bulawayo virus passed through donkey 2208).

1 horse was tested with O : Reaction.

1 horse, 1st tested with O, 2nd hyperimmunised with OTB, and 3rd hyperimmunised with OTBLPW : Reaction with the 1st test.

1 horse, tested with O, 2nd tested with Tzaneen, and 3rd hyperimmunised with O : No reaction.

Immunisation of Horses with Bulawayo Virus.

Test.						Test.					
Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.	Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
1906. Nov. 21	1938	c.c. 2	Ord.	62	R	1906. Dec. 7	2409	c.c. 9000	OTB LPW	1	—
Nov. 14	2401	9000	OTB	3	?						
1906. Nov. 21	2403	500	OTB	4	—	1906. Dec. 12	2416	8500	OTB LPW	2	—
1907. Jan. 22	2419	10	Tzn.	13	—	Jan. 29	2553	9000	Ord.	65	—

R—Reaction. ?—Doubtful.

Summary of Results of Tests on Horses Previously Immunised with Bulawayo Virus.

No. of Horses.	Tested with.	Result. Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Died.
2	Ordinary Virus	2	—	—	—
2	Tzaneen	—	—	—	1
4	Bulawayo	1	—	—	—
2	OTB	—	—	1	1
1	OTBLPW	—	—	—	—

Immunisation of Horses with Virus of Spontaneous and Relapse Cases.

Horse No.	Immunisation. Date of Injection.	Serum. Orig.	Virus. No.	Virus. Orig.	Virus. Gen.	Result.	Test. Date of Injection.	Virus. No.	Virus. Qu.	Virus. Orig.	Virus. Gen.	Result.
	1906.						1906.		c.c.			
1402	April 12	Ord.	1785	Turnbull	1	?	July 19	2058	20	Tzn.	6	R†
1586	Feb. 28	,,	1785	,,	1	?	May 10	1918	5000	Turnbull	6	—
							May 11	1937	3000	,,	6	—
1666	,,	,,	1785	,,	1	R	April 10	1901	9000	Ord.	41	—
1670	Mar. 24	,,	1785	,,	1	RD	May 10	1918	9000	Turnbull	6	—
1829	Mar. 29	,,	1785	,,	1	RD	,,	1938	9000	Ord.	62	—
1834	May 11	,,	1785	,,	1	R	June 15	1916	9000	,,	45	—
							Aug. 26	2090	9000	Tzn.	9	R
1837	Feb. 28	,,	1785	,,	1	RD	April 11	1901	9000	Ord.	41	—
1867	Mar. 23	,,	1785	,,	1	RD	June 8	2006	9000	,,	3	—
1868	,,	,,	1785	,,	1	RD	May 3	1893	9000	Turnbull	5	—
1872	Mar. 24	,,	1785	,,	1	R	May 10	1937	6000	,,	6	—
							May 18	1962	3000	,,	7	—
							Nov. 22	2403	6000	OTB	4	—
							Dec. 4	2418	3000	,,	5	—
1963	May 12	,,	1785	,,	1	R	June 30	2040	3000	Tzn.	4	RD
							July 5	2056	6000	,,	5	RD
							Dec. 7	1954	2	Bul.	1	—
							1906.					
1881	Mar. 23	,,	1788	Dale	1	R	May 11	1938	6000	Ord.	62	—
							,,	1937	3000	Turnbull	6	—
							1906.					
1860	May 11	,,	1772	Warmbaths	1	RD	June 30	2040	9000	Tzn.	4	R
							Dec. 13	1954	2	Bul.	1	—
1957	June 25	,,	1772	,,	1	R	Oct. 2	2298	9000	,,	11	—

R = Reaction. ?—Doubtful. RD—Reaction with Dikkop. R†—Reaction and Died.

Immunisation of Horses with Virus of Spontaneous and Relapse Cases.

Test.						Test.					
Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.	Date of Injection.	Virus. No.	Qu.	Orig.	Gen.	Result.
1907.		c.c.									
Feb. 14	2635	3000	Ord.	68	—						
,,	2636	6000	,,	68	—						
1906.											
July 6	2056	2500	Tzn.	5	R						
,,	2082	500	,,	8	R						
Aug. 24	2090	6000	,,	9	R						
						1906.		c.c.			
Aug. 3	1869	1	,,	1	—	Aug. 17	1964	1	Bul.	2	—
						1907.					
Sept. 18	2168	3000	Bul.	10	—	Jan. 26	2556	9000	OTB LPW-Spont. C.	1	—
Sept. 29	2225	6000	,,	3	—						
June 28	2026	3000	Tzn.	3	R	Feb. 14	2636	3000	Ord.	68	—
,,	2033	3000	,,	5	R	Feb. 20	2637	3000	,,	69	—
July 5	2056	2000	,,	5	R						
July 21	2060	10	Bul.	2	RD†						
July 6	2056	3000	Tzn.	5	RD†						
						1906.					
July 12	2060	9000	Bul.	2	RD	Sept. 4	2149	9000	Bul.	8	—
1907.											
Jan. 26	2599	6000	Spont.	1	—						
1906.											
Sept. 2	2152	3000	Tzn.	2	R	Nov. 21	1938	2	Ord.	62	—
Sept. 5	2151	6000	,,	10	R						
190 .											
Feb. 7 14	2635	3000	Ord.	68	—						
Feb. 19	2637	3000	,,	69	—						
1906.											
July 12	2060	6000	Bul.	2	RD†						
1906.						1906.					
Aug. 25	2090	6000	Tzn.	11	—	Nov. 21	1938	2	Ord.	62	R
Aug. 31	2152	3000	,,	2	—						
1907.											
Feb. 7	2625	6000	Ord.	66	—						

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop. RD†—Reaction Dikkop and Died.

Analysis from Preceding Table.

12 horses, immunised with virus Spontaneous Cases.

1 horse was twice hyperimmunised with O : No reaction.

1 horse, 1st hyperimmunised with O, and 2nd hyperimmunised with Tzaneen : Reaction when hyperimmunised with Tzaneen.

1 horse, 1st hyperimmunised with O, 2nd hyperimmunised with Tzaneen, and 3rd hyperimmunised with O : Reaction when hyperimmunised with Tzaneen.

1 horse, hyperimmunised with O, and 2nd tested with Bulawayo : Reaction, dikkop, and died.

1 horse, 1st hyperimmunised with O, 2nd tested with Tzaneen, 3rd tested with Bulawayo, 4th hyperimmunised with Tzaneen, 5th hyperimmunised with Bulawayo, and 6th hyperimmunised with Spont. C.–OTBLPW : Reaction when hyperimmunised with Tzaneen.

1 horse was tested with Tzaneen : Reaction and died.

1 horse, 1st hyperimmunised with Tzaneen, 2nd hyperimmunised with Tzaneen, 3rd tested with O, 4th tested with Bulawayo, and 5th hyperimmunised with O : Reaction and dikkop with 1st hyperimmunised Tzaneen, and reaction with 2nd hyperimmunised Tzaneen.

1 horse was hyperimmunised with Spont. C. : No reaction.

1 horse, 1st hyperimmunised with Spont. C., and 2nd hyperimmunised with O : No reaction.

1 horse, 1st hyperimmunised with Spont. C., and 2nd hyperimmunised with Tzaneen : Reaction, dikkop, and died.

1 horse, 1st hyperimmunised with Spont. C., 2nd and 3rd hyperimmunised with Bulawayo, 4th hyperimmunised with OTB, and 5th hyperimmunised with Spont. C. : Reaction and dikkop with 2nd hyperimmunisation.

1 horse, 1st hyperimmunised with $\left\{\begin{matrix}\text{Ord.}\\ \text{Spont.}\end{matrix}\right\}$ and 2nd hyperimmunised with Bulawayo : Reaction, dikkop, and died.

2 horses, immunised with virus Relapse Case.

1 horse, 1st and 2nd hyperimmunised with Tzaneen, 3rd tested with O, 4th tested with Bulawayo, and 5th hyperimmunised with O : Reaction with 1st hyperimmunised Tzaneen.

1 horse was hyperimmunised with Bulawayo : No reaction.

Summary of Results of Tests on Horses Previously Immunised with Virus of Spontaneous and Relapse Cases.

No. of Horses.	Tested with.	Result.			
		Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Died.
7	Ordinary Virus	—	—	—	—
6	Tzaneen	4	1	—	2
5	Bulawayo	—	1	—	2
1	OTB	—	—	—	—
4	Spontaneous Case	—	—	—	—
1	Ord.-Spont. C.	—	—	—	—
1	OTBLPW-Spont. C... ..	—	—	—	—

Conclusions.

These experiments show conclusively that when a horse or mule is inoculated with a certain strain of virus, the animal, as a rule, is immune against that particular strain, but when the animal is tested or hyperimmunised at a later date with virus of a different strain, reactions and deaths are noted, thus proving that the immunity afforded by the first inoculation is in no way complete.

The following table shows the percentage of reactions (including doubtful reaction and dikkop) and deaths amongst the inoculated mules and horses included in the previous experiments. This table is compiled according to the number of the test (1st, 2nd, 3rd, etc.), and irrespective of the virus with which they were immunised or tested, and will serve as an indication of the results to be expected in practice.

It is particularly instructive as demonstrating the difference between the immunity in horses and mules when obtained under the same conditions.

Percentage of Reactions and Deaths amongst Inoculated Horses and Mules when tested.

Number of Test.	Reactions.		Deaths.	
	Mules.	Horses.	Mules.	Horses.
	Per cent.	Per cent.	Per cent.	Per cent.
1st	2·4	18·0	1·8	10·5
2nd	2·8	11·6	Nil.	5·2
3rd	1·9	10·4	0·7	1·6
4th	2·5	4·4	0·3	2·2
5th	1·2	3·9	0·3	2·2
6th	Nil.	2·9	Nil.	0·6
7th	Nil.	2·3	Nil.	0·6
8th	—	0·6	—	Nil.
Total ...	10·6	52·4	3·0	23·2

"F."—IMMUNISATION OF MULES WITH INADEQUATE AND ADEQUATE SERUM AND VIRUS, AND THE IMMUNITY OBTAINED THEREFROM.

Under the phrase "Adequate Serum" is understood a serum which has been obtained from a horse injected with a corresponding virus. For instance, Ordinary serum is the adequate serum to Ordinary virus. An inadequate simultaneous inoculation would therefore be an inoculation of, say, Ordinary serum and Tzaneen virus.

The experiments were undertaken for the purpose of noting the extent to which adequate and inadequate inoculations can be made with safety, and to comp with the immunity obtained in this way.

(See pages 89 and 90 for explanation of O, T, etc.)

A.—Inadequate Serum and Virus.

Experiment No. 1.—To note effect of injection of serum (O strain) together with virus Tzaneen (horse 1863), first generation).

(*a*) Serum 89/90 (2 parts horse serum 89 to 1 part mule serum 90). Dose 300 c.c., injected subcutaneously.

Virus 1869, dose 2 c.c., injected subcutaneously.

1. *Mule* 2155.—

Injected on 2nd August, 1906, with 300 c.c. serum, and immediately after with 2 c.c. virus.

Result.—Reaction 7 days later. Died on 16th August, 1906, from horse-sickness.

2. *Mule* 2158.—

Injected on 2nd August, 1906, with 2 c.c. virus, and immediately after with 300 c.c. serum.

Result.—Reaction 5 days later, lasting for 8 days.

Tested 1st September, 1906, with 5 c.c. virus 1964 Bulawayo. No reaction. Tested on 3rd January, 1907, 2 c.c. virus 2407 (horse 2407 Ord.), slight reaction. Hyperimmunised 13th September, 1907, virus mule 2266 Bulawayo. No reaction.

(*b*) Serum 89/90 (equal parts of horse and mule serum). Dose 400 c.c., injected subcutaneously.

Virus 1869, dose 2 c.c., injected subcutaneously.

3. *Mule* 2161.—

Injected on the 2nd August, 1906, with 400 c.c. serum mixture, and immediately after with 2 c.c. virus.

Result.—Reaction 8 days later, lasting until 18th August, 1906. Dikkop on 15th day.

Tested 1st September with 5 c.c. virus 1964 (Bulawayo). Atypical reaction. On 13th September hyperimmunised with virus mule 2266 (Bulawayo). No reaction. Tested on 3rd January, 1907, with virus horse 2407 Ord. Slight reaction.

4. *Mule* 2164.—

Injected on 2nd August, 1906, 2 c.c. virus 1869, and directly after with 400 c.c. serum mixture.

Result.—Incubation 7 days; reaction 9 days. Dikkop 17th day.

Tested 1st September with 5 c.c. virus 1964 (Bulawayo). Irregular reaction. Retested 16th October, 1906, with 20 c.c. virus horse 1938 (Ord.). Slight reaction.

Conclusion.—Ordinary serum (viz., serum of horses and mules hyperimmunised with the strain O of the station) when injected simultaneously and subcutaneously with virus T of a horse give a reaction typical for horse-sickness. This immunity is not complete, as a subsequent inoculation of either virus B or virus O caused slight reactions in some instances.

Experiment No. 2.—To note effect of injection of serum O together with virus Bulawayo (horse 2060, first generation).

(*a*) Serum 89/90 (2 parts of horse serum 89 to 1 part of mule serum 90). Dose 300 c.c., injected subcutaneously.

Virus 2060, dose 2 c.c., injected subcutaneously.

1. *Mule* 2156.—

Injected on 2nd August, 1906, with 300 c.c. serum mixture, and directly after subcutaneously with 2 c.c. virus.

Result.—Incubation 4 days; reaction 9 days; dikkop 17th day.

Tested on 1st September intrajugularly with 20 c.c. virus horse 1869 Tzaneen. No reaction. 13th September hyperimmunised with virus mule 2267 Tzaneen. No reaction. Retested 12th January, 1907, with 2 c.c. virus mule 2287 Ord. Typical and distinct reaction.

2. *Mule* 2159.—

Injected on 2nd August, 1906, 2 c.c. virus horse 2060, and directly after with 300 c.c. serum mixture.

Result.—Incubation 3 days; reaction lasted 8 days.

Tested on 1st September with 20 c.c. virus horse 1869 Tzaneen. Reaction.

(*b*) Serum 89/90 (horse and mule serum mixed in equal proportions). Dose 400 c.c., injected subcutaneously.

Virus 2060, dose 2 c.c., injected subcutaneously.

3. *Mule* 2162.

Injected on 2nd August, 1906, with serum mixture, and directly after subcutaneously with 2 c.c. virus 2060.

Result.—Incubation 5 days; dikkop 11th day. Died 13th day.

4. *Mule* 2119.—

Injected on 2nd August, 1906, with virus 2060 subcutaneously, and directly after with serum mixture.

Result.—Incubation 4 days; lasted 10 days; dikkop 16th day.

Tested on 1st September by injection of 20 c.c. virus horse 1869 Tzaneen. Irregular slight reaction. On the 13th September hyperimmunised with virus mule 2267 (T). Slight reaction. Retested on 12th January, 1907, with mule 2287 Ord. Typical and distinct reaction.

Conclusion.—Ordinary serum, viz., serum of horses and mules hyperimmunised with O strain of the station, permits, when injected with virus B, a reaction typical for horse-sickness. This immunity is not complete, as a subsequent inoculation of virus O or virus B caused reactions.

The example given of mule 2119 is interesting, inasmuch as it showed reactions to all three vira.

Experiment No. 3.—To note effect of injection of serum O together with a mixture of Bulawayo and Tzaneen virus.

(*a*) Serum 89/90 mixed in proportion of 2 parts horse serum 89 to 1 part of mule serum 90. Dose 300 c.c., injected subcutaneously.

Virus mixture 2060 and 1869. Dose 2 c.c., injected subcutaneously.

1. *Mule* 2157.—

Injected on 2nd August, 1906, with serum mixture, and directly after with virus mixture (1869 and 2060).

Result.—Reaction after 5 days; dikkop 12th day. Died 14th day.

2. *Mule* 2160.—

Injected on 2nd August, 1906, with virus mixture, and directly after with serum mixture.

Result.—Incubation time 4 days, lasting 9 days. Dikkop 15th day.

Tested on 1st September by injection of 20 c.c. virus horse 1938 (O). Slight irregular reaction. Retested on 16th October by injection of 20 c.c. virus 1938 O. Reaction.

(*b*) Serum 89 and 90 mixed in equal parts of horse and mule serum. Dose 400 c.c., injected subcutaneously.

Virus as above.

3. *Mule* 2163.—

Injected on 2nd August, 1906, with serum mixture, and directly after with virus mixture.

Result.—Incubation 4 days; died on 11th day.

4. *Mule* 2120. —

Injected on 2nd August, 1906, with virus mixture, and directly after with serum mixture.

Result.—Incubation 4 days, lasting 7 days.

Tested on 1st September with 20 c.c. virus 1938 O. No reaction.

Conclusion.—Ordinary serum of horses and mules hyperimmunised with O strain of the station has not the same preventive action against a mixture of the virus T and B strains. Of 4 animals, 2 died of horse-sickness. In one animal the immunity obtained did not protect completely against a subsequent inoculation of virus O.

Experiment No. 4.—To note preventive value of serum mixture 91/92 (serum of horses and mules hyperimmunised with Ord. virus) and virus origin Tzaneen, injected simultaneously.

(*a*) Injections on 18th August, 1906, subcutaneously with 400 c.c. of serum mixture 91 and 92.

Virus 1869 (Tzaneen); dose 2 c.c., injected subcutaneously.

1. *Mule* 2181.—

Injected as above.

Result.—Reaction.

Tested on 20th September with 5 c.c. virus 2083 B. Slight reaction. Retested on 4th January, 1907, with 2 c.c. virus mule 2287 O. Irregular reaction.

2. *Mule* 2182.—
   Injected as above.
   Result.—Reaction.
   Tested on 20th October with 5 c.c. virus 2083 B. Slight reaction. Retested on 4th January with 2 c.c. virus 2407 O. Slight reaction.

3. *Mule* 2183.—
   Injected as above.
   Result.—Reaction.
   Tested on 20th September with 5 c.c. virus 2083 B. Reaction. Retested on 3rd January with virus mule 2287 O. Irregular reaction.

4. *Mule* 2184.—
   Injected as above.
   Result.—Reaction and dikkop on 14th day.
   Tested on 20th September with 5 c.c. virus 2083 B. No reaction. Retested on 4th January, 1907, with 2 c.c. virus horse 2407 O. Typical and distinct reaction.

Conclusion.—The inoculation of 400 c.c. serum O of horses and mules simultaneously and subcutaneously with virus T of horse permits a reaction typical for horse-sickness. The immunity established by it does not prevent a subsequent reaction due to the injection of virus Bul. and Ord. strains; some animals showing reactions to each separate injection.

(*b*) Serum as above.
Virus 1965 Tz. (mule); dose 2 c.c.
Serum and virus injected subcutaneously and simultaneously.

5. *Mule* 2185.—
   Injected on 18th August, 1906, with 400 c.c. mixture of serum 91/92, and subcutaneously with 2 c.c. virus 1965.
   Result.—Reaction.
   Tested on 20th September by an injection of 5 c.c. virus horse 2083 Bulawayo. Reaction, dikkop and death.

6. *Mule* 2186.—
   Injected as above.
   Result.—Reaction.
   Tested on 20th September with 5 c.c. virus horse 2083 B. Reaction. Retested on 4th January with virus horse 2407 O. Distinct and typical reaction.

7. *Mule* 2187.—
   Injected as above.
   Result.—Reaction, with dikkop on the 14th day.
   Tested on 20th September with 5 c.c. virus horse 2083 B. Doubtful reaction. On 4th January, 1907, retested with 2 c.c. virus mule 2287 O. Reaction.

8. *Mule* 2188.
   Injected as above.
   Result.—Reaction.
   Tested on 20th September with 5 c.c. virus 2083 B. Slight reaction. Retested on 3rd January with 2 c.c. virus 2287 O. Slight reaction.

Conclusions.—The inoculation of mules with serum O and virus Tzaneen permits of a reaction typical for horse-sickness. The immunity obtained does not prevent a subsequent reaction due to the virus of Bul. and Ord. strains, all animals showing reactions, due to each injection of virus.

Experiment No. 5.—To note value of various sera in connection with virus origin Tzaneen on mules, when injected simultaneously.

(*a*) Serum mixture 91/92 of horses and mules hyper-immunised with Ord. virus. Dose 400 c.c., injected subcutaneously.

Virus 1965 Tz. Dose 2 c.c., injected subcutaneously.

1. *Argentine Mule* 2211.—

Injected on 30th August, 1906, with 400 c.c. serum mixture 91/92, and simultaneously and subcutaneously with 2 c.c. virus mule 1965 (origin Tzaneen).

Result.—Slight reaction.

Tested with 2 c.c. virus Tzaneen 1965, injected intrajugularly on 19th September, 1906. Slight reaction in typical time for horse-sickness. Retested on 20th November with 5 c.c. virus horse 1938 O. Reaction. Retested on 8th December with 5 c.c. virus horse 2199 T. No reaction.

2. *Argentine Mule* 2213.—

Injected as above.

Result.—Reaction after incubation time of 4 days, lasting 10 days; dikkop 14th day. Not tested.

3. *Argentine Mule* 2214.—

Injected as above.

Result.—Reaction after incubation time of 6 days, lasting 8 days.

Tested on 26th October with 5 c.c. virus horse 1938 O. Reaction and dikkop on 10th day. Exposed at Onderstepoort farm, near Pretoria, and contracted horse-sickness spontaneously, accompanied with dikkop, and recovered.

4. *Argentine Mule* 2215.—

Injected as above.

Result.—Reaction after 4 days' incubation, lasting 12 days.

Tested on 26th October with 5 c.c. virus 1938 O. Severe reaction.

Conclusion.—The inoculation of mules with serum O and virus Tzaneen permits of a reaction typical for horse-sickness. The immunity obtained does not prevent a subsequent reaction when injected with virus O.

The instance of mule 2211 is interesting, inasmuch as a subsequent injection of virus 1965 caused a reaction. Mule 2214 had shown dikkop three times within seven months, the last one naturally contracted.

(*b*) Serum mixture 93 and 94, of horses and mules, hyper-immunised with Ordinary virus. Dose 400 c.c., injected subcutaneously.

Virus 1965 Tz., dose 2 c.c., injected subcutaneously.

1. *Argentine Mule* 2216.—
   Injected on 30th August, 1906, with 400 c.c. serum mixture 93/94, simultaneously and subcutaneously with 2 c.c. virus mule 1965.
   Result.—A typical reaction.
   Tested on 19th September, 1906, with 2 c.c. virus 1965 injected into jugular vein. Slight but typical reaction. Retested on 20th November with 5 c.c. virus 1938 O. Reaction. Retested on 8th December with 5 c.c. virus horse 2199 T. No reaction.
2. *Argentine Mule* 2217.—
   Injected as above.
   Result.—Incubation time of 4 days; reaction 9 days.
   Tested on 26th October with 5 c.c. virus 1938 O. Pronounced reaction.
3. *Argentine Mule* 2218.—
   Injected as above.
   Result.—Incubation time of 6 days; reaction lasting 11 days.
   Tested on 26th October with 5 c.c. virus 1938 O. Reaction and dikkop on 10th day.
4. *Argentine Mule* 2219.—
   Injected as above.
   Result.—Incubation time of 5 days; reaction lasted 10 days.
   Tested on 26th October with 5 c.c. virus 1938 O. Reaction.

   Conclusions.—The inoculation of serum O and virus Tzaneen permits of a reaction for horse-sickness. The immunity obtained does not prevent a subsequent reaction due to the injection of virus O.

Experiment No. 6.—To note effect of Ordinary serum of various dates when injected 24 hours previous to virus Tzaneen or virus Bulawayo.

(*a*) Serum 23/24, dated 11/7/05. Dose 400 c.c.

1. *Argentine Mule* 2220.—
   Injected on 30th August, 1906, with 400 c.c. serum 23/24 and 24 hours later with 2 c.c. virus mule 1964 (origin Bulawayo).
   Result.—Reaction after 5 days' incubation. Dikkop on 11th day. Died on 13th day.
2. *Argentine Mule* 2221.—
   Injected with 400 c.c. serum 23/24, and 24 hours later with 2 c.c. virus mule 1965 (origin Tzaneen).
   Result.—Incubation time of 3 days. Reaction lasted 9 days.
   Tested on 26th October with 5 c.c. virus horse 1938 O. Reaction; dikkop on 8th day, and died from horse-sickness on 10th day.

(*b*) Serum 75/76 (dated 8th January, 1908). Dose 400 c.c.

3. *Argentine Mule* 2222.—
   Injected on 30th August, 1906, with 400 c.c. serum 75/76, and 24 hours later with 2 c.c. virus mule 1964 (Bulawayo).
   Result.—Incubation time of 5 days; reaction for 10 days.
   Tested on 26th October with 5 c.c. virus 1938 O. Reaction.

4. *Argentine Mule* 2223.

Injected on 30th August, 1906, with 400 c.c. serum 75/76, and 24 hours later with 2 c.c. virus mule 1965 (Tzaneen).

Result.—Slight reaction.

Tested on 19th September, 1906, with 2 c.c. virus mule 1965, injected intrajugularly. Slight reaction. Retested on 20th November, 1906, with 5 c.c. virus mule 2287 O. Reaction. Retested on 8th December with 5 c.c. virus horse 2199 Tzaneen. Reaction.

Conclusions.—The injection of serum about 1 year old, 24 hours previous to injection of virus Bulawayo and virus T did not prevent a horse-sickness reaction, death resulting from B virus. Serum of about 6 months old when injected in the same way permitted a reaction. The immunity obtained from Tzaneen virus did not prevent a reaction due to a subsequent injection of O virus, neither did the immunity obtained from virus B.

The immunity obtained from 1965 did not protect against the same strain 2199 (12th) generation.

Experiment No. 7.—To test serum mixture against virus Tzaneen (mule first generation and mule second generation; virus of a horse injected into a mule).

(*a*) Serum Nos. 93/94, dose 400 c.c. injected subcutaneously. Virus 1965 Tzaneen; dose 2 c.c. injected subcutaneously.

Note.—All mules 15 hands high.

1. *Mule* 2288.—

Injected on 3rd October, 1906, with 400 c.c. serum mixture subcutaneously and 2 c.c. virus 1965 mule (first generation) subcutaneously. Reaction, 6 days' incubation, lasting 10 days.

Tested on 26th October, 1906, with 5 c.c. virus O 1938 into jugular vein; reaction.

2. *Mule* 2294.—

Injected as above, on 3rd October, 1906. Very slight reaction.

Tested on 26th October, 1906, with 5 c.c. virus O 1938 intrajugularly. Reaction and developed dikkop on 8th day. Died on the 18th day from horse-sickness.

(*b*) Virus 1995 Tzaneen (second generation mule). Serum as above.

3. *Mule* 2291.—

Injected on the 3rd October, 1906, with 400 c.c. serum 93/94 and subcutaneously with 2 c.c. virus 1995 (Tzaneen mule, second generation). Reaction after six days incubation, lasting 6 days; dikkop 16th day.

Tested on 26th October, 1906, with 5 c.c. virus O 1938 intrajugularly. Reaction.

4. *Mule* 2293.—

Injected as above, on 3rd October, 1906. Reaction after 8 days' incubation, lasting 8 days. Dikkop on 13th day.

Tested on 31st October, 1906, with 5 c.c. virus O 1938 intrajugularly. Slight reaction.

Conclusions.—The injection of serum O and Tzaneen virus permitted horse-sickness reactions. The immunity obtained did not prevent reactions due to subsequent injections of virus O.

(*c*) Serum mixture 95/96. Dose 400 c.c. injected subcutaneously.

Virus 1965 Tzaneen (mule, first generation). Dose 2 c.c. injected subcutaneously.

5. *Mule* 2289.—

Injected on 3rd October, 1906, with 400 c.c. serum 95/96 and 2 c.c. virus 1965 (Tzaneen mule, first generation). Very slight reaction.

Tested on 25th October, 1906, with 5 c.c. virus 1938 O. Reaction.

6. *Mule* 2290.—

Injected as above on 3rd October, 1906.

Result.—Slight reaction.

Tested on 25th October, 1906, with 5 c.c. virus O 1938 injected intrajugularly. Reaction.

(*d*) Virus Tzaneen (mule, second generation). Serum as above.

7. *Mule* 2292.—

Injected on 3rd October, 1906, with 400 c.c. serum 95/96 and 2 c.c. virus 1995 (Tzaneen mule, second generation).

Result.—Slight reaction.

Tested on 25th October, 1906, with 5 c.c. virus O 1938 injected intrajugularly. Reaction.

8. *Mule* 2212.—

Injected as above, on 3rd October, 1906.

Result.—Slight reaction.

Tested on 25th October, 1906, with 5 c.c. virus O 1938 intrajugularly. Reaction.

Conclusion.—The injection of serum O and virus T permitted a horse-sickness reaction. The immunity obtained did not prevent a reaction due to a subsequent inoculation of virus O.

Experiment No. 8.—Serum tests of various compositions (O strain).

(*a*) Virus Tzaneen, horse 2199 (12th generation). Dose 2 c.c. Serum mixture of Nos. I., II. and III. (of horses hyperimmunised once to three times) and ordinary mule serum (mules hyperimmunised once to three times); mixture No. 102; dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously

1. *Argentine Mule* 2315.—

Injected as above.

Result.—Incubation 5 days, reaction lasting 9 days.

Tested on 21st December with 2 c.c. virus 1938 O. Reaction.

2. *Argentine Mule* 2316.—

Injected as above.

Result.—Incubation 4 days; died on 9th day of horse-sickness.

3. *Argentine Mule* 2317.—
   Injected as above. Second injection on 7th day of 200 c.c. same serum.
   Result.—Incubation 5 days; died on 9th day.

4. *Argentine Mule* 2318.—
   Injected as above. Second injection on 7th day with 200 c.c. same serum.
   Result.—Incubation 6 days; died on the 10th day.

   Conclusion.—The injection of O serum simultaneously and subcutaneously with virus Tzaneen of horse (12th generation) resulted in a mortality of 3 animals out of 4—75 per cent. The immunity obtained in the one surviving animal did not prevent a reaction due to a subsequent injection of virus O.

(*b*) Virus Tzaneen, horse 2199 (12th generation). Dose 2 c.c. Serum mixture of horse serum No. 4 (horses hyperimmunised four times) and mule serum 104 (1, 2 and 3) mixed in equal quantities.

Virus and serum injected subcutaneously and simultaneously.

5. *Argentine Mule* 2319.—
   Injected as above on the 18th October, 1906. Second injection on the 7th day with 200 c.c. of the same serum.
   Result.—Incubation 5 days; dikkop on the 11th day Died on the 12th day.

6. *Argentine Mule* 2320.—
   Treated as above. Second injection of serum on the 7th day.
   Result.—Incubation 5 days; reaction lasting 12 days.
   Tested on the 7th December with 5 c.c. mule virus 2295 (Tzaneen horse virus 2199 passed through mule) Slight reaction. Retested on 18th December with 2 c.c. virus 1938 (Ordinary virus). Reaction.

7. *Argentine Mule* 2321.—
   Injected as above, but no second injection.
   Result.—Incubation 5 days; reaction lasted 6 days.
   Tested on 7th December with 5 c.c. virus 2295 (Tzaneen). Slight reaction. Retested on 18th December with 2 c.c. virus 1938 Ordinary. Slight reaction.

8. *Argentine Mule* 2322.—
   Injected as above, and second injection on the 7th day with 200 c.c. same serum.
   Result.—Incubation 5 days; reaction 9 days.
   Tested on 12th January, 1907, with 2 c.c. virus mule 2287 O. Reaction.

9. *Argentine Mule* 2323.—
   Injected as above, but no second injection.
   Result.—Incubation 5 days; died on the 11th day.

10. *Argentine Mule* 2324.—
    Injected as above, no second injection.
    Result.—Incubation 5 days; reaction on the 11th day.

Tested on the 7th December with 5 c.c. virus 2295 Tzaneen; slight reaction. Retested on 18th December with 2 c.c. virus 2287, Ordinary, mule. No reaction.

Conclusion.—Serum O of horses hyperimmunised 4 times and of mules 1—3 times hyperimmunised permitted a typical reaction of horse-sickness with virus 2199 Tzaneen (12th generation). The immunity obtained did not prevent reactions to subsequent injections of virus of the same strain passed through a mule, nor against a subsequent inoculation of virus O.

(*c*) Virus 2199 (Tzaneen horse 12th generation); dose 2 c.c. Serum: Mixture in equal parts of serum 101 (horses hyperimmunised 1 to 3 times) and of mule serum (mules hyperimmunised 1 to 3 times).

Virus and serum injected subcutaneously and simultaneously.

11. *Argentine Mule* 2325.—
Injected as above.
Result.—Incubation 5 days; reaction 9 days; dikkop on the 17th day.
Tested on the 12th January, 1907, with 2 c.c. virus 2287 O. Reaction.

12. *Argentine Mule* 2326.—
Injected as above.
Result.— 5 days' incubation; reaction lasted 8 days; dikkop on 12th day.
Tested on the 12th January with 2 c.c. virus 2287 O. Reaction.

13. *Argentine Mule* 2327.—
Injected as above; second injection of 200 c.c. same serum on the 7th day.
Result.—Incubation 5 days; reaction lasted 8 days.
Tested on 7th December, 1906, with 5 c.c. virus 2295 Tzaneen. Slight reaction. Retested on 18th December, 1906, with 2 c.c. virus 2287 (Ordinary virus mule). Distinct reaction.

14. *Argentine Mule* 2328.—
Injected as above; second injection on 7th day with 200 c.c. same serum.
Result.—Incubation 4 days; reaction 9 days.
Tested on 7th December with 5 c.c. virus 2295 (Tzaneen). Slight reaction. Retested on 18th December with 2 c.c. virus 2287 (Ordinary). Reaction.

Conclusion.—The injection of serum O simultaneously and subcutaneously with virus Tzaneen (12th generation) permitted of typical horse-sickness reactions. The immunity obtained did not prevent a reaction due to a subsequent injection of virus of the same strain or of the O strain.

(*d*) Virus 2199 (horse Tzaneen 12th generation). Dose 2 c.c. Serum: A mixture of serum 95 (horses hyperimmunised 1 to 3 times) and serum 96 (mules hyperimmunised 1 to 3 times) in equal parts. . Dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously.

15. *Mule* 2329.—
Injected as above.
Result.—Incubation 5 days; reaction lasting 7 days.
Tested on 7th December, 1906, with 5 c.c. virus 2295 (Tzaneen); reaction. Retested on 18th December, 1906, with 2 c.c. virus 1938 (Ordinary). Doubtful reaction.

16. *Mule* 2330.—
Injected as above, and second injection of 200 c.c. serum on the 7th day.
Result.—Incubation 5 days; reaction lasting 8 days.
Tested on 7th December, 1906, with 5 c.c. virus 2295 (Tzaneen); slight reaction. Retested on the 22nd December, 1906, with 2 c.c. virus 2287 (Ordinary mule); reaction.

17. *Mule* 2331.—
Injected as above; second injection of 200 c.c. same serum on 7th day.
Result.—Incubation 5 days; reaction 6 days.
Tested on the 20th November, 1906, with 2 c.c. virus 1938 (horse, ordinary); slight reaction. Retested on the 8th December, 1906, with 5 c.c. virus 2295 (Tzaneen, mule); no reaction.

18. *Mule* 2332.—
Injected as above.
Result.—Incubation 5 days; died on the 10th day.
Conclusion.—The simultaneous injection of serum O and virus Tzaneen 2199 (12th generation) resulted in a typical horse-sickness reaction. In two instances the immunity obtained did not prevent a reaction due to the subsequent injection of the same strain of virus, nor a reaction due to the O virus.

(*c*) Virus 2199 (horse Tzaneen, 12th generation); dose 2 c.c. Serum Nos. 97/98, mixed in equal parts (horses and mules hyperimmunised 1 to 3 times).
Virus and serum injected subcutaneously and simultaneously.

19. *Mule* 2333.—
Injected as above on the 18th October, 1906; second injection of 200 c.c. of same serum on the 7th day.
Result.—5 days' incubation; reaction lasting 7 days; dikkop on the 11th day.
Tested on the 20th November, 1906, with 2 c.c. virus O 1938 (horse). Reaction. Retested on the 8th December, 1906, with 5 c.c. virus 2295 (Tzaneen, mule); no reaction.

20. *Mule* 2334.—
Injected as above; no second injection of serum.
Result.—Incubation 5 days; dikkop on the 10th day; died on the 14th day.

21. *Mule* 2335.—
Injected as above; second injection of 200 c.c. same serum on the 7th day.
Result.—4 days' incubation; reaction lasting 8 days.

Tested on 20th November, 1906, with 5 c.c. virus horse 1938 Ordinary; reaction. Retested on 8th December, 1906, with 5 c.c. virus horse 2295 (Tzaneen); slight reaction.

22. *Mule* 2336.—

Injected as above; no second injection.

Result.—5 days' incubation; reaction 6 days; dikkop on the 9th day.

Tested on the 20th November, 1906, with 5 c.c. virus 1938 Ordinary; no reaction. Retested on the 8th December, 1906, with 5 c.c. virus 2295 (Tzaneen); slight reaction.

Conclusion.—The simultaneous injection of serum O and virus T resulted in a horse-sickness reaction. In two cases the immunity obtained did not prevent a reaction to a subsequent injection of virus of the same strain, nor a reaction due to the injection of O strain.

(*f*) Virus 2199 (horse Tzaneen, 12th generation); dose 2 c.c. Serum 99/100 of horses and mules hyperimmunised 1 to 3 times; mixed in equal parts. Dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously. Date, 18th October, 1906.

23. *Mule* 2337.—

Injected as above; second injection of 200 c.c. same serum on the 7th day.

Result.—Incubation 5 days; reaction 7th day; dikkop on the 9th day.

Tested on the 20th November, 1906, with 2 c.c. virus 1938; reaction. Tested on the 8th December, 1906, with 5 c.c. virus 2295; no reaction.

24. *Mule* 2338.—

Injected as above; second injection of 200 c.c. same serum on the 7th day.

Result.—Incubation 5 days; reaction lasted 9 days.

Tested on the 20th November, 1906, with 2 c.c. virus 1938; slight reaction. Retested on the 8th December, 1906, with 5 c.c. virus 2295; slight reaction.

25. *Mule* 2339.—

Injected as above; no second injection of serum.

Result.—Incubation 5 days; reaction lasted 9 days; dikkop on the 11th day.

Tested on the 20th November, 1906, with 2 c.c. virus mule 2287 (Ordinary); reaction. Retested on the 8th December, 1906, with 5 c.c. virus mule 2295; slight reaction.

Conclusion.—The simultaneous injection of serum O and virus T (12th generation) resulted in a horse-sickness reaction. In three instances the immunity obtained did not prevent a reaction, due to a subsequent injection of virus of the same strain, and in three cases not against the injection of virus O.

(*g*) Virus 2199 (horse Tzaneen, 12th generation); dose 2 c.c. Serum mixture 103/104, in equal parts, of horses and mules hyperimmunised 1 to 3 times; dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously. Date, 18th October, 1906.

26. *Mule* 2341.—
Injected as above; second injection of 200 c.c. same serum on the 7th day.
Result.—5 days' incubation; reaction lasting 7 days.
Tested on 20th November, 1906, with 5 c.c. virus 2287; reaction. Retested on the 8th December, 1906, with 5 c.c. virus 2295; no reaction.

27. *Mule* 2342.—
Injected as above, but no second injection of serum.
Result.—Incubation 5 days; reaction 8 days.
Tested on the 20th November, 1906, with 2 c.c. virus 2287 into jugular vein; slight reaction.

28. *Mule* 2343.—
Injected as above; second injection of 200 c.c. same serum on 7th day.
Result.—Incubation 5 days; dikkop on the 11th day; died on the 13th day.

29. *Mule* 2344.—
Injected as above, but no second injection of serum.
Result.—Incubation 5 days; died on the 11th day.
Conclusion.—Simultaneous injection of serum O and virus Tzaneen (12th generation) resulted in reactions and two deaths. The immunity obtained did not prevent a reaction due to a subsequent injection of virus of the O strain.

(*h*) Virus 2199 (horse Tzaneen, 12th generation); dose 2 c.c. Serum mixture in equal parts of Nos. 105/106 of horses and mules hyperimmunised 1 to 3 times. Dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously. Date, 18th October, 1906.

30. *Mule* 2345.—
Injected as above; second injection of 200 c.c. same serum on the 7th day.
Result.—5 days' incubation; reaction 8 days; dikkop on the 12th day.
Tested on the 20th November, 1906, with 2 c.c. virus 2287 O; reaction. Retested on the 8th December, 1906, with 5 c.c. virus 2295 T; reaction.

31. *Mule* 2346.—
Injected as above, but no second injection of serum.
Result.—Incubation 4 days; reaction 11 days.
Tested on the 20th November, 1906, with 5 c.c. virus 2287 O; reaction. Retested on the 8th December, 1906, with 5 c.c. virus 2295 T; reaction.

32. *Mule* 2347.—
Injected as above; second injection of 200 c.c. serum on the 7th day.
Result.—Incubation 5 days; reaction 8 days; dikkop on the 11th day.

Tested on the 20th November, 1906, with 5 c.c. virus 2287; no reaction. Retested on the 8th December, 1906, with 5 c.c. virus 2295; no reaction.

33. *Mule* 2348.—
Injected as above, but no second injection of serum.
Result.—Incubation 5 days; dikkop on the 10th day; died on the 12th day.
Conclusion.—The simultaneous injection of serum O and virus T (12th generation) resulted in horse-sickness reactions. The immunity obtained in two out of three cases did not prevent a reaction to a subsequent inoculation of virus of the same strain, nor to the O strain.

Experiment No. 9.—With serum O strain of various compositions, and virus Tzaneen, first generation.
(*a*) Virus 1965 Tzaneen mule, first generation; dose 2 c.c. Serum mixture 107/108 mixed in equal parts of horses and mules hyperimmunised 1 to 3 times. Dose 400 c.c.
Virus and serum injected subcutaneously and simultaneously.

1. *Argentine Mule* 2362.
Injected as above on the 1st November, 1906.
Result.—Slight reaction.
Tested on the 8th December, 1906, with 5 c.c. virus O mule 2287; reaction.

2. *Argentine Mule* 2374.—
Injected as above.
Result.—Slight reaction; died on the 16th November, 1906; dikkop on the 13th day.

3. *Argentine Mule* 2375.—
Injected as above.
Result.—Reaction after 6 days' incubation, lasting for 10 days.
Tested on the 8th December, 1906, subcutaneously with 5 c.c. virus O 2287 mule. Reaction.
Conclusion.—The simultaneous injection of serum O and virus Tzaneen mule (first generation) resulted in horse-sickness reactions. Two animals when tested later with virus O showed reactions.
(*b*) Virus 1965 Tzaneen mule (first generation); dose 2 c.c. Serum mixture 109/110 in equal parts (of horses and mules hyperimmunised 1 to 3 times). Dose 400 c.c.
Virus and serum injected subcutaneously and simultaneously. Date, 1st November, 1906.

4. *Argentine Mule* 2370.—
Injected as above.
Result.—Slight reaction.
Tested on the 18th December, subcutaneously with 2 c.c. virus 2287; slight reaction.

5. *Argentine Mule* 2365.—
Injected as above.
Result.—Very slight reaction.
Tested on the 18th December, 1906, subcutaneously with 2 c.c. virus 2199; severe reaction. Retested on the

12th January, 1907, with 2 c.c. virus mule 2287 O. Slight reaction.

6. *Argentine Mule* 2366.—
Injected as above.
Result.—Slight reaction.
Tested on the 18th December, 1906, with 2 c.c. virus 2199 (Tz. horse 12th generation); reaction. Tested on the 12th January with 2 c.c. virus 2287 O; reaction.

Conclusion.—The simultaneous injection of serum O and the virus Tzaneen (first generation) (mule virus 4 months old), resulted in but very slight reactions. When tested subsequently with virus of the same strain (12th generation), reactions—and even severe reactions—were noted. The immunity obtained did not prevent reactions due to subsequent injections of virus O.

(*c*) Virus 1965; Tzaneen mule (first generation); dose 2 c.c. Serum mixture 111/112 in equal parts (of horses and mules hyperimmunised 1 to 3 times). Dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously. Date, 1st November, 1906.

7. *Argentine Mule* 2368.—
Injected as above.
Result.—Doubtful reaction, if any.
Tested on the 18th December, 1906, with 2 c.c. virus 2287 O; distinct reaction.

8. *Argentine Mule* 2369.—
Injected as above.
Result.—Slight reaction.
Tested on the 18th December, 1906, with 2 c.c. virus 2287 O; injected subcutaneously; reaction, and died of horse-sickness on the 28th December, 1906.

9. *Argentine Mule* 2364.—
Injected as above.
Result.—Doubtful reaction.
Tested on the 18th January, 1906, with 2 c.c. virus 2199 (Tzaneen 12th generation); reaction, and died of dikkop on the 9th day—28th December, 1906.

Conclusion.—The simultaneous injection of serum O. and virus T (first generation mule) resulted in but slight or doubtful reactions. One animal subsequently tested with virus of the same strain (12th generation) died; of two tested with O strain, one had a severe reaction and recovered, one dying.

It is probable that the virus 1965 had become attenuated.

Experiment No. 10.—With serum O strain of various compositions and virus Tzaneen (second generation).

(*a*) Virus 1996, Tzaneen mule, second generation. Dose 2 c.c.

Serum mixture 113/114 in equal parts (of horses and mules hyperimmunised 1 to 3 times). Dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously. Date, 1st November, 1906.

1. *Argentine Mule* 2371.—
Injected on the 1st November, 1906, with 400 c.c. serum 113/114 and 2 c.c. virus mule 1996 subcutaneously.

Result.—Doubtful reaction.

Tested on the 18th December, 1906, subcutaneously with 2 c.c. virus T 2199 (12th generation). Result: Died of horse-sickness on the 30th December, 1906.

2. *Argentine Mule* 2372.—

Injected as above.

Result.—Doubtful reaction.

Tested on the 18th December, 1906, subcutaneously with 2 c.c. virus Tzaneen 2199 (12th generation); reaction; died on the 2nd January, 1907, of horse-sickness.

3. *Argentine Mule* 2367.—

Injected as above.

Result.—Doubtful reaction.

Tested on the 18th December, 1906, subcutaneously with 2 c.c. virus 1965; reaction; died on 8th January, 1907.

Conclusion.—The simultaneous injection of serum O and virus 1996 T resulted in a doubtful reaction, if any at all. There was a slight disturbance, but apparently no trace of immunity was caused by it. All three mules died, when subsequently injected with virus of the same strain, 12th generation.

(*b*) Virus 1996, Tzaneen mule, second generation. Dose 2 c.c.

Serum mixture 115/116 in equal parts of horses and mules hyperimmunised 1 to 3 times. Dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously. Date, 1st November, 1906.

4. *Argentine Mule* 2363.—

Injected with 400 c.c. serum 115/116 and 2 c.c. virus mule 1996 (Tzaneen).

Result.—Doubtful reaction.

Tested on the 18th December, 1906, subcutaneously with 2 c.c. virus 1965; reaction; died on the 5th January, 1907.

5. *Argentine Mule* 2382.—

Injected as above.

Result.—Doubtful reaction.

Tested on the 8th December, 1906, by subcutaneous injection of 5 c.c. virus O 1938; severe reaction; died of horse-sickness on the 22nd December, 1906.

6. *Argentine Mule* 2379.—

Injected as above.

Result.—Doubtful reaction.

Tested on the 8th December, 1906, subcutaneously with 5 c.c. virus 1938 O (62nd generation); severe reaction; recovered.

Conclusion.—The simultaneous injection of serum O and virus 1996 T strain, resulted in a doubtful reaction; but little immunity was caused by it. A subsequent inoculation of virus O caused death in two animals, and a severe reaction in a third one.

(*c*) Virus 1965 Tz. mule, first generation. Dose 2 c.c.
Serum mixture Nos. I., II. and III. and mule 112. Dose 400 c.c.
Virus and serum injected subcutaneously and simultaneously.
Date, 1st November, 1906.

7. *Argentine Mule* 2376.—
Injected with 400 c.c. serum subcutaneously and simultaneously with 2 c.c. virus 1965.
Result.—Reaction; dikkop on the 21st November, 1906.
Tested on the 8th December, 1906, with 5 c.c. virus mule 2287; slight reaction.
(*d*) Virus 1996 Tz. mule, 2nd generation. Dose 2 c.c.
Serum as above.
Date, 1st November, 1906.

8. *Argentine Mule* 2383.—
Injected as above.
Result.—Doubtful reaction.
Tested on the 8th December, 1906, subcutaneously with 5 c.c. virus 1938 O; severe reaction; died on the 20th December, 1906, of horse-sickness, with dikkop.
Conclusion.—The simultaneous inoculation of a mule with virus Tzaneen 1965, mule 1st generation and serum O, resulted in a typical horse-sickness reaction; the immunity obtained still permitted a reaction due to a subsequent inoculation of virus O.
The simultaneous inoculation of serum O and virus 1996 gave a doubtful reaction, and no immunity was obtained against a subsequent inoculation of virus O strain.

Analysis of Results.

Deaths.

38 mules immunised with virus Tzaneen, 1st generation...	2 deaths.
9 mules immunised with virus Tzaneen, 2nd generation (attenuated or inert virus)	0 deaths.
34 mules immunised with virus Tzaneen, 12th generation	10 deaths.
	12 deaths.
6 mules immunised with virus Bulawayo, 2nd generation	2 deaths.
4 mules immunised with a mixture of virus Tzaneen 1st generation, and Bulawayo 2nd generation	2 deaths.

Tests.

(a) *Tests with Ordinary Virus.*

18 *mules immunised with Tzaneen, 1st generation.*

12 tested with Ordinary, 62nd generation: 8 reactions, 2 reactions and dikkop, 2 deaths.
6 tested with Ordinary, 38th generation: 5 reactions and 1 death.

5 *mules immunised with Tzaneen, 2nd generation (attennated or inert virus).*
Tested with Ordinary, 62nd generation: 3 reactions and 2 deaths.

5 *mules immunised with Tzaneen, 12th generation*
4 tested with Ordinary, 38th generation: 4 reactions.
1 tested with Ordinary, 62nd generation: 1 reaction.

1 *mule immunised with a mixture of virus of Tzaneen 1st generation and Bulawayo 2nd generation.*
Tested with Ordinary, 62nd generation: No reaction.

1 *mule immunised with a mixture of virus of Tzaneen 1st generation and Bulawayo 2nd generation.*
1st test with Ordinary, 62nd generation: Doubtful reaction.
2nd test with Ordinary, 62nd generation: Distinct reaction.

1 *mule immunised with Bulawayo, 2nd generation.*
Tested with Ordinary, 62nd generation: Reaction.

NOTE.—1 mule (2214) which was immunised with Tzaneen, 1st generation, and had a reaction with dikkop, again showed a reaction and dikkop with test of Ordinary, 62nd generation, and later contracted horse-sickness spontaneously, showing a third reaction and dikkop.

(b) *Tests with Tzaneen Virus.*

1 *mule immunised with Tzaneen, 1st generation.*
Tested with Tzaneen, 12th generation: Died.

4 *mules immunised with Tzaneen, 2nd generation (inert or attenuated virus).*
2 tested with Tzaneen, 1st generation: 2 reactions.
2 tested with Tzaneen, 12th generation: 2 deaths.

1 *mule immunised with Bulawayo, 2nd generation.*
Tested with Tzaneen, 1st generation: Reaction.

(c) *1st Test with Ordinary and 2nd Test with Tzaneen Virus.*

12 *mules immunised with Tzaneen, 12th generation.*
6 tested with Ordinary, 62nd generation, and Tzaneen, 13th generation:
2 reactions on both tests.
3 reactions with Ordinary.
1 reaction with Tzaneen.
6 tested with Ordinary, 38th generation, and Tzaneen, 13th generation:
4 reactions on both tests.
1 reaction with Ordinary.
1 no reaction.

(d) *1st Test with Tzaneen and 2nd Test with Ordinary Virus.*

7 *mules immunised with Tzaneen, 12th generation.*
3 tested with Tzaneen, 13th generation, and Ordinary, 62nd generation:
2 reactions on both tests.
1 distinct reaction with Tzaneen and a doubtful reaction with Ordinary.

4 tested with Tzaneen, 13th generation, and Ordinary, 38th generation:
3 reactions on both tests.
1 reacted only with Tzaneen.

2 *mules immunised with Tzaneen, 1st generation.*
Tested with Tzaneen, 12th generation, and Ordinary, 38th generation: Reactions on both tests.

(e) *1st Test with Tzaneen, 2nd Test with Ordinary, and 3rd Test with Tzaneen Virus.*

3 *mules immunised with Tzaneen, 1st generation.*
1 mule tested with Tzaneen, 1st generation, Ordinary, 38th generation, and Tzaneen, 12th generation: Reaction on all three tests.
2 mules tested with Tzaneen, 1st generation, Ordinary, 62nd generation, and Tzaneen, 12th generation: reaction only with first and second tests.

(f) *1st and 2nd Test with Tzaneen and 3rd Test with Ordinary Virus.*

2 *mules immunised with Bulawayo, 2nd generation.*
Tested with Tzaneen, 1st generation, Tzaneen, 3rd generation, and Ordinary, 38th generation:
1 reaction on each test; 1 had only a reaction with Ordinary.

(g) *1st Test with Bulawayo and 2nd Test with Ordinary Virus.*

9 *mules immunised with Tzaneen, 1st generation.*
1 mule tested with Bulawayo, 2nd generation, and Ordinary, 62nd generation:
Doubtful reaction with the 1st, and distinct reaction with the 2nd test.
8 mules tested with Bulawayo, 4th generation, and Ordinary, 38th generation:
3 reactions on both tests.
2 distinct reactions with 1st, and a doubtful reaction with the 2nd test.
1 doubtful reaction with the 1st, and a distinct reaction with the 2nd test.
1 reaction only with the 2nd test.
1 died on the 1st test (Bulawayo).

(h) *1st and 2nd Test with Bulawayo and 3rd Test with Ordinary Virus.*

2 *mules immunised with Tzaneen, 1st generation.*
Tested with Bulawayo, 2nd generation, Bulawayo, 3rd generation, and Ordinary, 38th generation:
1 gave a reaction on the 3rd test.
1 showed a doubtful reaction to 1st Bulawayo test, and a distinct reaction with Ordinary 3rd test.

1 *mule was not tested.*

Tabulated Résumé of Previous Experiments.
Inadequate Serum and Virus.

Mule.	Immunisation.				I. Test.			II. Test.			III. Test.		
	Serum.	Virus.		Result.	Virus.		Result.	Virus.		Result.	Virus.		Result.
	Orig.	Orig.	Gen.		Orig.	Gen.		Orig.	Gen.		Orig.	Gen.	
2155	Ord.	Tzn.	1	R†			—	—	—	—	—	—	—
2158	,,	,,	1	R	Bul.	2	—	Bul.	3	—	Ord.	38	R
2161	,,	,,	1	RD	,,	2	?	,,	3	—	,,	38	R
2164	,,	,,	1	RD	,,	2	?	Ord.	62	R	—	—	—
2156	,,	Bul.	2	RD	Tzn.	1	—	Tzn.	3	—	Ord.	38	R
2159	,,	,,	2	R	,,	1	R	—	—	—	—	—	—
2162	,,	,,	2	RD†	—	—	—	—	—	—	—	—	—
2119	,,	,,	2	RD	Tzn.	1	R	Tzn.	3	R	Ord.	38	R
2157	,,	Tzn. Bul.	1 2	RD†	—	—	—	—	—	—	—	—	—
2160	,,	,,	1 2	RD	Ord.	62	?	Ord.	62	R	—	—	—
2163	,,	,,	1 2	R†	—	—	—	—	—	—	—	—	—
2120	,,	,,	1 2	R	Ord.	62	—	—	—	—	—	—	—
2181	,,	Tzn.	1	R	Bul.	4	R	Ord.	38	?	—	—	—
2182	,,	,,	1	R	,,	4	R	,,	38	R	—	—	—
2183	,,	,,	1	R	,,	4	R	,,	38	?	—	—	—
2184	,,	,,	1	RD	,,	4	—	,,	38	R	—	—	—
2185	,,	,,	1	R	,,	4	RD†	—	—	—	—	—	—
2186	,,	,,	1	R	,,	4	R	Ord.	38	R	—	—	—
2187	,,	,,	1	RD	,,	4	?	,,	38	R	—	—	—
2188	,,	,,	1	R	,,	4	R	,,	38	R	—	—	—
2211	,,	,,	1	R	Tzn.	1	R	,,	62	R	Tzn.	12	—
2213	,,	,,	1	RD	Not Tested.			—	—	—	—	—	—
2214	,,	,,	1	RD	Ord.	62	RD	Later a 3rd Dikkop Spont.					
2215	,,	,,	1	R	,,	62	R	—	—	—	—	—	—
2216	,,	,,	1	?	Tzn.	1	R	Ord.	62	R	Tzn.	12	—
2217	,,	,,	1	R	Ord.	62	R	—	—	—	—	—	—
2218	,,	,,	1	R	,,	62	RD.	—	—	—	—	—	—
2219	,,	,,	1	R	,,	62	R	—	—	—	—	—	—
2220	,,	Bul.	2	RD†	—	—	—	—	—	—	—	—	—
2222	,,	,,	2	R	Ord.	62	R	—	—	—	—	—	—
2221	,,	Tzn.	1	R	,,	62	RD†	—	—	—	—	—	—
2223	,,	,,	1	R	Tzn.	1	R	Ord.	38	R	Tzn.	12	R
2288	,,	,,	1	R	Ord.	62	R	—	—	—	—	—	—
2294	,,	,,	1	R	,,	62	RD†	—	—	—	—	—	—
2291	,,	,,	2	RD	,,	62	R	—	—	—	—	—	—
2293	,,	,,	2	RD	,,	62	R	—	—	—	—	—	—
2289	,,	,,	1	R	,,	62	R	—	—	—	—	—	—
2290	,,	,,	1	R	,,	62	R	—	—	—	—	—	—
2292	,,	,,	1	R	,,	62	R	—	—	—	—	—	—
2212	,,	,,	1	R	,,	62	R	—	—	—	—	—	—
2315	,,	,,	12	R	,,	62	R	—	—	—	—	—	—
2316	,,	,,	12	R†	—	—	—	—	—	—	—	—	—
2317	,,	,,	12	R†	—	—	—	—	—	—	—	—	—
2318	,,	,,	12	R†	—	—	—	—	—	—	—	—	—

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop. R†—Reaction and died. RD†—Reaction with Dikkop and died.

TABULATED RÉSUMÉ OF PREVIOUS EXPERIMENTS—(*Continued*).

INADEQUATE SERUM AND VIRUS—(*Continued*).

Mule.	Immunisation.				I. Test.			II. Test.			III. Test.		
	Serum.	Virus.		Result.	Virus.		Result.	Virus.		Result.	Virus.		Result.
	Orig.	Orig.	Gen.		Orig.	Gen.		Orig.	Gen.		Orig.	Gen.	
2319	Ord.	Tzn.	12	RD†	—	—	—	—	—	—	—	—	—
2320	,,	,,	12	R	Tzn.	13	R	Ord.	62	R	—	—	—
2321	,,	,,	12	R	,,	13	R	,,	62	R	—	—	—
2322	,,	,,	12	R	Ord.	38	R	—	—	—	—	—	—
2323	,,	,,	12	R†	—	—	—	—	—	—	—	—	—
2324	,,	,,	12	R	Tzn.	13	R	Ord.	38	—	—	—	—
2325	,,	,,	12	RD	Ord.	38	R	—	—	—	—	—	—
2326	,,	,,	12	RD	,,	38	R	—	—	—	—	—	—
2327	,,	,,	12	R	Tzn.	13	R	Ord.	38	R	—	—	—
2328	,,	,,	12	R	,,	13	R	,,	38	R	—	—	—
2329	,,	,,	12	R	,,	13	R	,,	62	?	—	—	—
2330	,,	,,	12	R	,,	13	R	,,	38	R	—	—	—
2331	,,	,,	12	R	Ord.	62	R	Tzn.	13	—	—	—	—
2332	,,	,,	12	R†	—	—	—	—	—	—	—	—	—
2333	,,	,,	12	RD	Ord.	62	R	Tzn.	13	—	—	—	—
2334	,,	,,	12	RD†	—	—	—	—	—	—	—	—	—
2335	,,	,,	12	R	Ord.	62	R	Tzn.	13	R	—	—	—
2336	,,	,,	12	RD	,,	62	—	,,	13	R	—	—	—
2337	,,	,,	12	RD	,,	62	R	,,	13	—	—	—	—
2338	,,	,,	12	R	,,	62	R	,,	13	R	—	—	—
2339	,,	,,	12	RD	,,	38	R	,,	13	R	—	—	—
2340	,,	,,	12	R	,,	38	R	,,	13	R	—	—	—
2341	,,	,,	12	R	,,	38	R	,,	13	—	—	—	—
2342	,,	,,	12	R	,,	38	R	—	—	—	—	—	—
2343	,,	,,	12	RD†	—	—	—	—	—	—	—	—	—
2344	,,	,,	12	R†	—	—	—	—	—	—	—	—	—
2345	,,	,,	12	RD	Ord.	38	R	Tzn.	13	R	—	—	—
2346	,,	,,	12	R	,,	38	R	,,	13	R	—	—	—
2347	,,	,,	12	RD	,,	38	—	,,	13	—	—	—	—
2348	,,	,,	12	RD†	—	—	—	—	—	—	—	—	—
2362	,,	,,	1	R	Ord.	38	R	—	—	—	—	—	—
2374	,,	,,	1	RD†	—	—	—	—	—	—	—	—	—
2375	,,	,,	1	R	Ord.	38	R	—	—	—	—	—	—
2370	,,	,,	1	R	,,	38	R	—	—	—	—	—	—
2365	,,	,,	1	R	Tzn.	12	R	Ord.	38	R	—	—	—
2366	,,	,,	1	R	,,	12	R	,,	38	R	—	—	—
2368	,,	,,	1	?	Ord.	38	R	—	—	—	—	—	—
2369	,,	,,	1	R	,,	38	R†	—	—	—	—	—	—
2364	,,	,,	1	?	Tzn.	12	RD†	—	—	—	—	—	—
2371	,,	,,	2	?	,,	12	R†	—	—	—	—	—	—
2372	,,	,,	2	?	,,	12	R†	—	—	—	—	—	—
2367	,,	,,	2	?	,,	1	R	—	—	—	—	—	—
2363	,,	,,	2	?	,,	1	R	—	—	—	—	—	—
2382	,,	,,	2	?	Ord.	62	R†	—	—	—	—	—	—
2379	,,	,,	2	?	,,	62	R	—	—	—	—	—	—
2376	,,	,,	1	RD	,,	38	R	—	—	—	—	—	—
2383	,,	,,	2	?	,,	62	RD†	—	—	—	—	—	—

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop. R†—Reaction and died.
RD†—Reaction with Dikkop and died.

Complete Summary of Results.

Tests on mules previously immunised with virus Tzaneen 1 gen.

Mules.	Tested with Virus.	Result.			
		Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Deaths.
10	Ordinary 38 gen. ..	8	—	2	1
20	Ordinary 62 gen. ..	18	2	—	2
3	Bulawayo 2 gen. ..	—	—	2	—
2	Bulawayo 3 gen. ..	—	—	—	—
8	Bulawayo 4 gen. ..	5	—	1	1
3	Tzaneen 1 gen. ..	3	—	—	—
5	Tzaneen 12 gen. ..	3	—	—	1

Tests on mules previously immunised with virus Tzaneen 2 gen.

Mules.	Tested with Virus.	Result.			
		Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Deaths.
5	Ordinary 62 gen. ..	3	—	—	2
4	Tzaneen 12 gen. ..	2	—	—	2

Tests on mules previously immunised with virus Tzaneen 12 gen.

Mules.	Tested with Virus.	Result.			
		Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Deaths.
14	Ordinary 38 gen. ..	12	—	—	—
10	Ordinary 62 gen. ..	8	—	1	—
19	Tzaneen 13 gen. ..	14	—	—	—

Tests on mules previously immunised with virus Bulawayo 2 gen. :

Mules.	Tested with Virus.	Result.			
		Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Deaths.
2	Ordinary 38 gen. ..	2	—	—	—
1	Ordinary 62 gen. ..	1	—	—	—
3	Tzaneen 1 gen.	2	—	—	—
2	Tzaneen 3 gen.	1	—	—	—

Tests on mules previously immunised with a mixture of virus { *Tzaneen 1 gen.* / *Bulawayo 2 gen.* }

Mules.	Tested with Virus.	Result.			
		Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Deaths.
2	Ordinary 62 gen. ..	1	—	1	—

B.—Adequate Serum and Virus.

Experiment No. 11.—With serum mixture 119 O strain injected subcutaneously; dose 400 c.c. and 2 c.c. virus horse 2407 O strain. Serum and virus injected subcutaneously and simultaneously on 8th January, 1907.

1. *Mule* 2566.—
   Immunised as above.
   Result.—Slight reaction.
   Tested on the 22nd January with 2 c.c. same virus: no reaction.
2. *Mule* 2567.—
   Immunised as above.
   Result.—Slight reaction.
   Tested on the 22nd January with same virus: no reaction.
3. *Mule* 2568.—
   Immunised as above.
   Result.—Distinct reaction.
   Tested on the 22nd January with same virus; no reaction.
4. *Mule* 2569.—
   Immunised as above.
   Result.—Slight reaction.
   Tested on the 22nd January with same virus; no reaction.
5. *Mule* 2570.—
   Immunised as above.
   Result.—Distinct reaction.
   Tested on the 22nd January with same virus; no reaction.
6. *Mule* 2571.—
   Immunised as above.
   Result.—Distinct reaction.
   Tested on the 22nd January; no reaction.
7. *Mule* 2572.—
   Immunised as above.
   Result.—Distinct reaction.
   Tested on the 22nd January; no reaction.
8. *Mule* 2573.—
   Immunised as above.
   Result.—Reaction.
   Tested on the 22nd January; no reaction.

9. *Mule* 2574.—
Immunised as above.
Result.—Slight reaction; dikkop on the 14th day.
Tested on the 22nd January, same virus; no reaction.

10. *Mule* 2575.—
Immunised as above.
Result.—Slight reaction.
Tested on the 22nd January; no reaction.

11. *Mule* 2576.—
Immunised as above.
Result.—Reaction.
Tested on the 22nd January; no reaction.

12. *Mule* 2577.—
Immunised as above.
Result.—Reaction.
Tested on the 22nd January, same virus; no reaction.

Conclusion.—The simultaneous inoculation of serum O and virus O 2407 resulted in typical horse-sickness reactions. The immunity obtained proved to be complete to a subsequent inoculation of the same virus.

Experiment No. 12.—With serum mixture 119 O strain; dose 300 c.c. and virus 2287 mule O strain; dose 2 c.c.
Virus and serum injected subcutaneously and simultaneously.
Date, 12th January, 1907.

1. *Mule* 2525.—
Immunised as above.
Result.—Distinct reaction.
Tested on the 21st February with 2 c.c. virus mule 2268 T; distinct reaction. Retested on the 13th March, 2 c.c. virus 1954 B; distinct reaction.

2. *Mule* 2526.—
Immunised as above.
Result.—Slight reaction.
Not tested.

3. *Mule* 2527.—
Immunised as above.
Result.—Slight reaction.
Not tested.

Conclusion.—The simultaneous inoculation of serum O and virus O (38th generation) was succeeded by typical horse-sickness reactions. The immunity obtained was tested in one instance, and proved not to be able to prevent a subsequent reaction due to the injection of Tzaneen virus and Bulawayo virus.

Experiment No. 13.—With serum mixture No. 121 (O strain).
Dose 300 c.c.; virus 2287 O strain (38th generation mule).
Dose 2 c.c., virus and serum injected subcutaneously and simultaneously.
Date, 12th January, 1908.

1. *Mule* 2528.—
Immunised as above.
Result.—Reaction and dikkop.

Tested on the 21st February with 2 c.c. virus 1954 B; reaction and dikkop on the 13th day. Retested on the 13th March with 2 c.c. virus mule 2268 T; no reaction.

2. *Mule* 2529.—

Immunised as above.
Result.—Reaction and dikkop on the 14th day.
Tested on the 21st February with virus mule 2268 T; reaction. Retested on the 13th March with 2 c.c. virus 1954 B; no reaction.

3. *Mule* 2530.—

Immunised as above.
Result.—Reaction and dikkop on the 13th day.
Not tested.

Conclusion.—The simultaneous inoculation of serum O and virus O resulted in typical reactions. When tested subsequently, in the first instance with Bulawayo virus, a reaction ensued, and the immunity now established protected against a subsequent inoculation of Tzaneen strain. In the second instance reaction was noticed due to a subsequent inoculation of Tzaneen virus, and the immunity established afterwards protected against an injection of Bulawayo strain.

Experiment No. 14.—With serum mixture No. 123 O strain, and virus mule 2287 O strain.

Dose of serum 300 c.c., and dose of virus 2 c.c.
Virus and serum injected subcutaneously and simultaneously.
Date, 12th January, 1907.

1. *Mule* 2531.—

Immunised as above.
Result.—Reaction.
Not tested.

2. *Mule* 2532.—

Immunised as above.
Result.—Reaction.
Tested on the 21st February with virus 1954 B: reaction, dikkop and death on the 18th day.

3. *Mule* 2533.—

Immunised as above.
Result.—Reaction and dikkop on the 14th day.
Not tested.

Conclusion.—The simultaneous inoculation of serum O and virus of the same strain resulted in typical horse-sickness reactions. One of the mules was tested with virus of Bulawayo strain, when it contracted horse-sickness and died at an unusual late date after inoculation.

Experiment No. 15.—With serum mixture No. 125 O strain and virus mule 2287 O strain.

Dose of serum 300 c.c., and dose of virus 2 c.c.
Serum and virus injected subcutaneously and simultaneously.
Date, 18th January, 1907.

1. *Mule* 2508.—
   Immunised as above.
   Result.—Slight reaction.
   Not tested.
2. *Mule* 2509.—
   Immunised as above.
   Result.—Slight reaction.
   Not tested.
3. *Mule* 2511.—
   Immunised as above.
   Result.—Reaction.
   Not tested.
4. *Mule* 2512. —
   Immunised as above.
   Result.—Reaction.
   Not tested.

Conclusion.—Typical reactions due to simultaneous inoculation of serum O and virus O.

Experiment No. 16.—With serum mixture No. 127 O strain and virus mule 2287 O strain.

Serum dose 300 c.c., virus dose 2 c.c.
Serum and virus injected subcutaneously and simultaneously.
Date, 18th January, 1907.

1. *Mule* 2534.—
   Injected as above.
   Result.—Doubtful reaction.
2. *Mule* 2535.—
   Injected as above.
   Result.—Slight reaction.
3. *Mule* 2536.—
   Injected as above.
   Result.—Slight reaction.
4. *Mule* 2537.—
   Injected as above.
   Result.—Slight reaction.

Conclusion.—Typical reactions due to simultaneous inoculation of serum O and virus O.

Experiment No. 17.—With serum mixture No. 129 O strain and virus mule 2287 O strain.

Serum dose 300 c.c., virus dose 2 c.c.
Serum and virus injected subcutaneously and simultaneously.
Date, 18th January, 1907.

1. *Mule* 2538.—
   Immunised as above.
   Result.—Slight reaction.
   Not tested.
2. *Mule* 2540.—
   Immunised as above.
   Result.—Distinct reaction.
   Not tested.

3. *Mule* 2541.—
Immunised as above.
Result.—Reaction.
Not tested.
4. *Mule* 2542.—
Immunised as above.
Result.—Reaction.
Not tested.
Conclusion.—Typical reactions due to simultaneous inoculation of O serum and O virus.

Experiment No. 18.—With serum mixture No. 131 O strain and virus mule 2287 O strain.
Serum dose 300 c.c., virus dose 2 c.c.
Serum and virus injected subcutaneously and simultaneously.
Date, 18th January, 1907.
1. *Mule* 2543.—
Immunised as above.
Result.—Reaction.
Not tested.
2. *Mule* 2544.—
Immunised as above.
Result.—Reaction.
Not tested.
3. *Mule* 2545.—
Immunised as above.
Result.—Reaction.
Not tested.
4. *Mule* 2546.—
Immunised as above.
Result.—Reaction.
Not tested.
Conclusion.—Typical reactions due to simultaneous inoculation of O serum and O virus.

Experiment No. 19.—With serum mixture No. 133 O strain and virus mule 2287 O strain.
Serum dose 300 c.c., virus dose 2 c.c.
Serum and virus injected subcutaneously and simultaneously.
Date, 18th January, 1907.
1. *Mule* 2547.—
Immunised as above.
Result.—Reaction.
Not tested.
2. *Mule* 2548.—
Immunised as above.
Result.—Distinct reaction.
Not tested.
3. *Mule* 2549.—
Immunised as above.
Result.—Reaction and died of horse-sickness on the 10th day.
Conclusion.—Typical reactions due to simultaneous inoculation of O serum and O virus.

Experiment No. 20.—With serum mixture No. 135 O strain and virus mule 2287 O strain.

Serum dose 300 c.c., virus dose 2 c.c.

Serum and virus injected subcutaneously and simultaneously.

Date, 22nd January, 1907.

1. *Mule* 2578.—

Result.—Reaction, dikkop on the 10th day, and death on the 11th day from horse-sickness.

2. *Mule* 2579.—

Immunised as above.

Result.—Distinct reaction.

Not tested.

3. *Mule* 2580.—

Immunised as above.

Result.—Distinct reaction.

Not tested.

Conclusion.—Typical reactions due to simultaneous inoculation of serum O and virus O.

Experiment No. 21.—With serum mixture No. 137 O strain and virus mule 2287 O strain.

Serum dose 300 c.c., virus dose 2 c.c.

Serum and virus injected subcutaneously and simultaneously.

Date, 22nd January, 1907.

1. *Mule* 2582.—

Immunised as above.

Result.—Reaction, dikkop on the 10th day, and died on the 11th day.

2. *Mule* 2583.—

Immunised as above.

Not tested.

Result.—Reaction.

3. *Mule* 2584.—

Immunised as above.

Result.—Reaction.

Not tested.

Conclusion.—Typical reactions due to simultaneous inoculation of serum O and virus O.

Experiment No. 22.—With serum mixture No. 139 O strain and virus mule 2287 O strain.

Serum dose 300 c.c., virus dose 2 c.c.

Serum and virus injected subcutaneously and simultaneously.

Date, 22nd January, 1907.

1. *Mule* 2585.—

Injected as above.

Result.—Distinct reaction.

2. *Mule* 2586.—

Injected as above.

Result.—Slight reaction.

3. *Mule* 2587.—

Injected as above.

Result.—Slight reaction.

Conclusion.—Typical reactions due to simultaneous inoculation of serum O and virus O.

Tabulated Résumé from Previous Experiments.

Adequate Serum and Virus.

Mule.	Immunisation.				I. Test.			II. Test		
	Serum.	Virus.		Result.	Virus.		Result.	Virus.		Result.
	Orig.	Orig.	Gen.		Orig.	Gen.		Orig.	Gen.	
2566	Ord.	Ord.	38	R	Ord.	38	—	—	—	—
2567	,,	,,	38	R	,,	38	—	—	—	—
2568	,,	,,	38	R	,,	38	—	—	—	—
2569	,,	,,	38	R	,,	38	—	—	—	—
2570	,,	,,	38	R	,,	38	—	—	—	—
2571	,,	,,	38	R	,,	38	—	—	—	—
2572	,,	,,	38	R	,,	38	—	—	—	—
2573	,,	,,	38	R	,,	38	—	—	—	—
2574	,,	,,	38	RD	,,	38	—	—	—	—
2575	,,	,,	38	R	,,	38	—	—	—	—
2576	,,	,,	38	R	,,	38	—	—	—	—
2577	,,	,,	38	R	,,	38	—	—	—	—
2525	,,	,,	38	R	Tzn.	1	R	Bul.	1	R
2526	,,	,,	38	R	—	—	—	—	—	—
2527	,,	,,	38	R	—	—	—	—	—	—
2528	,,	,,	38	RD	Bul.	1	RD	Tzn.	1	—
2529	,,	,,	38	RD	Tzn.	1	R	Bul.	1	—
2530	,,	,,	38	RD	—	—	—	—	—	—
2531	,,	,,	38	R	—	—	—	—	—	—
2532	,,	,,	38	R	Bul.	1	RD†	—	—	—
2533	,,	,,	38	RD	—	—	—	—	—	—
2508	,,	,,	38	R	—	—	—	—	—	—
2509	,,	,,	38	R	—	—	—	—	—	—
2511	,,	,,	38	R	—	—	—	—	—	—
2512	,,	,,	38	R	—	—	—	—	—	—
2534	,,	,,	38	?	—	—	—	—	—	—
2535	,,	,,	38	R	—	—	—	—	—	—
2536	,,	,,	38	R	—	—	—	—	—	—
2537	,,	,,	38	R	—	—	—	—	—	—
2538	,,	,,	38	R	—	—	—	—	—	—
2540	,,	,,	38	R	—	—	—	—	—	—
2541	,,	,,	38	R	—	—	—	—	—	—
2542	,,	,,	38	R	—	—	—	—	—	—
2543	,,	,,	38	R	—	—	—	—	—	—
2544	,,	,,	38	R	—	—	—	—	—	—
2545	,,	,,	38	R	—	—	—	—	—	—
2546	,,	,,	38	R	—	—	—	—	—	—
2547	,,	,,	38	R	—	—	—	—	—	—
2548	,,	,,	38	R	—	—	—	—	—	—
2549	,,	,,	38	R†	—	—	—	—	—	—
2578	,,	,,	38	RD†	—	—	—	—	—	—
2579	,,	,,	38	R	—	—	—	—	—	—
2580	,,	,,	38	R	—	—	—	—	—	—
2582	,,	,,	38	RD†	—	—	—	—	—	—
2583	,,	,,	38	R	—	—	—	—	—	—
2584	,,	,,	38	R	—	—	—	—	—	—
2585	,,	,,	38	R	—	—	—	—	—	—
2586	,,	,,	38	R	—	—	—	—	—	—
2587	,,	,,	38	R	—	—	—	—	—	—

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop. R†—Reaction and died. RD†—Reaction with Dikkop and died.

Analysis of Results.

Deaths.

49 mules immunised with virus Ordinary, 38th generation; 3 deaths.

Tests.

A.—*Tests with Ordinary Virus.*

12 mules tested with Ordinary, 38th generation, gave no results.

B.—*Tests with Bulawayo Virus.*

1 mule tested with Bulawayo, 1st generation, had reaction, dikkop, and died.

C.—*1st Test with Bulawayo and 2nd Test with Tzaneen Virus.*

1 mule tested with Bulawayo, 1st generation, and afterwards with Tzaneen, 1st generation, had reaction and dikkop with 1st test and no reaction from the 2nd test.

D.—*1st Test with Tzaneen and 2nd Test with Bulawayo Virus.*

2 mules tested with Tzaneen, 1st generation, and later with Bulawayo, 1st generation:

1 mule reacted with both tests and
1 mule only with 1st test (Tzaneen).

30 mules were not tested.

Inadequate and Adequate Serum and Virus.

Résumé of Conclusions.

1. The simultaneous injection of serum O and adequate virus resulted in typical horse-sickness reactions, and the immunity obtained therefrom prevented a reaction when the adequate virus is subsequently reinjected.

2. The immunity obtained by a reaction after injection of serum O and virus O (adequate serum and virus) does not prevent a reaction due to a subsequent inoculation of virus of a different strain (Tzaneen and Bulawayo).

3. The simultaneous injection of serum O and virus Tzaneen (inadequate serum) is succeeded by reactions which proved not to be more fatal to animals than that due to virus O (adequate serum).

4. The immunity obtained by this reaction was in no way complete. It did not prevent a reaction either due to a subsequent inoculation of a different strain (O or Bulawayo), nor did it prevent reactions when the same strain of Tzaneen virus of a later generation was utilised.

5. The fact that a reaction was noted due to the subsequent inoculation of virus in an animal immunised with the same strain of virus can be explained either by accepting that the test virus, being of a higher generation and derived from horses, is of a greater virulency, or that the virus Tzaneen is already of a complex nature containing certain constituents of the O virus which are deviated by the O serum and accordingly during the immunisation leave no impression on the system of the animal. A subsequent inoculation of the same strain would then not meet the corresponding antibodies and a reaction would result.

6. In some of the animals a single injection of virus, together with adequate or inadequate serum, produced complete immunity against subsequent inoculations, hence a factor in the animal has also something to do with the creation of immunity.

7. In the foregoing experiments it has been noted that a virus can attenuate and completely lose its virulency.

8. Virus T of the 12th generation has increased enormously in virulency, which shows itself in the immunisation and in the tests.

"G."—INOCULATION OF MULES WITH POLYVALENT VIRUS AND SERUM.

Under the term "polyvalent serum" in this article is understood either a serum which is composed of various monovalent sera, obtained by mixing them together, or by a serum obtained from a horse previously injected with a mixture of three or more vira.

The object of the experiments was to determine whether such a polyvalent serum can be utilised with greater advantage in practice, and if the immunity afforded by the polyvalent virus would be a better protection against horse-sickness than that given by a monovalent virus.

Serum O-T-B.—This is a mixture made on the 5th November, 1906, and consisting of serum O (that is of horses immunised with the virus hitherto used on this station), serum T strain (of virus obtained from the Tzaneen Estate, Zoutpansberg), and serum B or Bulawayo (of horses hyperimmunised with a strain of virus obtained from Bulawayo).

(See also explanations on pages 89 and 90.)

Experiment No. 1.—On the 6th November, 1906, 400 c.c. serum O-T-B to be injected simultaneously and subcutaneously with virus O-T-B respectively of mules 2287 and 2268 and horse 2083.

1. Mule 2388.—
   Injected as above.
   Result.—A slight reaction.
   Tested on its immunity on the 27th November, with 10 c.c. virus 2268 Tzaneen strain, injected intrajugularly. On the 7th December the same dose was repeated. Slight reaction, the character of which was, however, doubtful. On the 18th December 2 c.c. virus mule 2287 strain O was injected subcutaneously. No reaction due to this injection.
2. *Mule* 2381.—
   Injected as above.
   Result.—A slight but distinct typical reaction ensued.
   Tested for immunity on the 27th November with 10 c.c. Bulawayo virus horse 2083, injected into jugular vein. This dose was repeated on the 7th December. There was a reaction which might be due to the injection of the 27th November, but which was not typical. Injected on the 18th December with 2 c.c. virus mule 2268 Tzaneen strain. There was an atypical reaction.

3. *Mule* 2377.—

Injected as above.

Result.—Hardly any reaction noticeable.

Tested.—On the 27th November and 7th December with 10 c.c. virus 2287 strain O. There was a reaction which might be due to the injection of the 27th November. On the 18th December 2 c.c. virus 2268 Tzaneen strain was subcutaneously injected. No reaction ensued.

Conclusion.—The immunity obtained from the virus mixture O-T-B protected the animals against any individual strain of which the virus was composed.

Experiment No. 2.—With serum as above, injected simultaneously and subcutaneously.

Dose 400 c.c. with virus mixture O-T, viz., mule 2287 and mule 2268.

1. *Mule* 2378.—

Injected as above on the 6th November.

Result.—There was a slight and typical reaction.

Tested for its immunity on the 27th November by an intrajugular injection of 10 c.c. virus same mixture; on the 7th December injected with 10 c.c. virus mule 2268 strain Tzaneen. There was a slight reaction, which may be due to the injection on the 27th November. On the 18th December injected subcutaneously with 2 c.c. virus mule 2287 Ordinary strain. No reaction after this injection.

2. *Mule* 2385.—

Injected as above.

Result.—A short atypical reaction was noticed.

Tested on the 8th December by an intrajugular injection of 10 c.c. virus of the same mixture. A reaction took place not typical for horse-sickness. On the 18th December injected subcutaneously with 2 c.c. virus 2199 strain Tzaneen. A reaction typical for horse-sickness took place, although the diagnosis could not be verified by clinical examination. On the 12th January, 1907, the animal was subcutaneously injected with 2 c.c. virus 2287 strain O. Reaction, but not typical for horse-sickness.

3. *Mule* 2386.—

Injected as above.

Result.—There was no noticeable reaction due to this injection.

Tested for immunity on the 27th November by intrajugular injection of 10 c.c. virus same mixture, and this dose was repeated on the 7th December. There was a slight reaction which was probably due to the injection of the 27th November. On the 18th December an injection of 2 c.c. virus 1938 Ordinary strain was made. No reaction.

Conclusion.—The immunity obtained from the mixture O-T virus protected the mules against the subsequent injection of the same virus, and also against its components, but one

animal showed a reaction, which would indicate that the protection was not complete

4. *Mule* 2384.—
   Injected as above.
   Result.—Typical horse-sickness reaction.
   Tested on the 27th November with intrajugular injection of 10 c.c. virus of the same mixture. The dose was repeated on the 7th December. A reaction ensued, probably due to the injection on the 27th November. On the 16th December this mule showed symptoms of dikkop, and died two days later.
5. *Mule* 2387.—
   Injected as above.
   Result.—A slight reaction took place.
   Tested on the 8th December by intrajugular injection of 10 c.c. virus of same mixture. An atypical reaction was noted. On the 18th December an injection of 2 c.c. virus 2199 Tzaneen strain was made. Again a slight reaction was noted. On the 12th January, 1907, a further injection of 2 c.c. virus Ordinary strain took place. This injection was also followed by a reaction.
6. *Mule* 2380.—
   Injected as above.
   Result.—Hardly any reaction noticeable.
   Tested on the 27th November with 10 c.c. virus of same mixture injected into the jugular vein. This dose was repeated on the 7th December. There was a slight reaction, but it was not typical. On the 18th December the animal was injected with 2 c.c. virus 2287 Ordinary strain. Again a reaction, which was atypical.

The immunity obtained through the inoculation of the virus O-T did not protect mule 2384 against the same virus mixture, and it died of horse-sickness. The other two animals also showed reactions, indicating that the immunity was not complete.

Conclusion.—By the simultaneous inoculation of a polyvalent serum composed of three different sera in equal quantities in conjunction with (*a*) the simultaneous injection of a mixture of the three corresponding virus, and (*b*) a mixture of two virus, an immunity was, generally speaking, established, but this immunity was not complete against subsequent inoculations of the same mixture, or of the components of the mixture. It must therefore be concluded that the immunity was only obtained against one component of the mixture, which immunity is, however, sufficient in the majority of cases to protect the animal against death due to another strain of virus.

Experiment No. 3.—Serum mixture of (1) mules hyperimmunised with Ordinary virus, (2) of horses hyperimmunised with Ordinary virus, (3) of horses hyperimmunised with Tzaneen virus, and (4) horses hyperimmunised with Bulawayo virus, mixed in equal quantities and injected simultaneously and subcutaneously with a virus mixture O-T-B.

(1*a*) Virus mixture of mules, viz., mule 2287 Ordinary virus, mule 2268 Tzaneen virus, and mule 1954 Bulawayo virus.
Dose of serum 400 c.c.

1. *Mule* 2467.—
   Injected as above, on the 5th December, 1906.
   Result.—A slight reaction.
   Tested on the 23rd January, 1907, with 2 c.c. virus of same mixture. An atypical reaction took place.

2. *Mule* 2466.—
   Injected as above.
   Result.—A distinct reaction.
   Tested with 2 c.c. virus mixture O-T-B of horses 2407 (Ordinary), 1869 (Tzaneen), and 2359 (Bulawayo). Typical reaction ensued, and the animal died on the 2nd February, 1907, from horse-sickness.

3. *Mule* 2472.—
   Injected as above.
   Result.—A distinct and typical reaction ensued.
   Tested on the 23rd January, with a mixture O-T-B of mule virus. An atypical reaction.

4. *Mule* 2474.—
   Injected as above.
   Result.—A slight reaction was noticed.
   Tested on the 23rd January with a mixture O-T-B of horse virus. A typical reaction, and mule died of horse-sickness on the 3rd February.

5. *Mule* 2468.—
   Injected as above.
   Result.—Reaction.
   Tested on the 23rd January with virus horse 2418. This virus is called OTB, obtained from the horse 2418 which was injected simultaneously with virus O horse 1938, with virus T horse 2199, and with virus B mule 1964. No reaction noted.

6. *Mule* 2471.—
   Injected as above.
   Result.—A distinct and typical reaction ensued.
   Tested on the 23rd January by subcutaneous injection of 2 c.c. virus 2418 OTB. A reaction ensued due to this inoculation.

7. *Mule* 2475.—
   Injected as above.
   Result.—A typical reaction ensued, from which it recovered. The animal died on the 12th January, 1907, of debility.

Conclusion.—The inoculation of the mixture of mule virus of the three various strains produced a reaction, and accordingly immunity had to be expected. When tested with virus of the same mixture, only a slight reaction took place, thus proving that the required immunity was sufficient to protect the animal against the subsequent inoculation. When tested with horse virus of the same strains two animals

succumbed. When tested with OTB virus of one horse the immunity proved sufficient, one animal showing a slight reaction.

(1*b*) Serum and virus as above.
Dose of serum 300 c.c.

8. *Mule* 2473.—
   Injected as above on the 5th December, 1906, with virus O of mule 2287, T of mule 2268, and B of mule 1954.
   Result.—There was a slight reaction.
   Tested on the 23rd January, 1907, with 2 c.c. virus subcutaneously injected of the same mixture. There was again a reaction.
9. *Mule* 2461.—
   Injected as above.
   Result.—Reaction.
   Tested on the 23rd January by injection separately of the three different virus. Nothing particular noted.
10. *Mule* 2464.—
   Injected as above.
   Result.—Distinct reaction.
   Tested on the 23rd January with virus 2418. Nothing particular was noted.
11. *Mule* 2470.—
   Injected as above on the 5th December.
   Result.—Reaction.
   Tested on the 23rd January by an injection of 2 c.c. virus horse 2406. This virus was derived from a horse which had been injected with virus O, with virus T, with virus B, and a virus of immunised mules which in practice had relapses. There were in addition to the foregoing mules one from Lydenburg, one from Piet Retief, one from Warmbaths, and two from Tzaneen (Altenroxel); this virus is called OTBLPW. There was no reaction due to this test.
12. *Mule* 2450.—
   Injected as above.
   Result.—Reaction.
   Tested on the 3rd January with 2 c.c. virus of mule 2287 Ordinary strain. There was a slight reaction—not certain whether due to this injection. On the 23rd January tested again by 2 c.c. virus mule 1954 Bulawayo strain; no reaction.
13. *Mule* 2448.—
   Injected as above on the 5th December.
   Result.—Reaction.
   Tested on the 3rd January with 2 c.c. virus mule 2268 Tzaneen strain; reaction not typical. Tested again on the 23rd January with 2 c.c. virus mule 2287 Ordinary strain. There was a retarded reaction, not likely due to this injection.
14. *Mule* 2469.—
   Injected as above on the 5th December.

Result.—Reaction.

Tested on the 3rd January with 2 c.c. virus mule 1954 Bulawayo strain; a retarded reaction, probably not due to this injection. On the 23rd January tested with 2 c.c. virus mule 2268 Tzaneen strain. Doubtful horse-sickness reaction.

Conclusion.—The inoculation of O-T-B virus protected against a subsequent inoculation of the same virus, of virus OTBLPW, and against the constituents of the virus mixture. There were, however, reactions in some instances, not typical for horse-sickness, and therefore it is uncertain whether they were produced by the test virus.

11*a*.—Virus mixture of horses (2407 O; 1869 Tz.; 1959 B). Serum as before, but dose of 400 c.c.

15. *Mule* 2449.—

Injected on the 5th December with 400 c.c. serum and 2 c.c. of above virus mixture.

Result.—Slight reaction.

Tested on the 23rd January with 2 c.c. virus mixture O-T-B of mule 2287 Ord., mule 2268 Tzaneen, and mule 1954 Bulawayo. Doubtful reaction.

16. *Mule* 2447.—

Injected as above.

Result.—Distinct reaction.

Tested on the 23rd January by an injection of a mixture of the same virus with which it was immunised. Distinct reaction.

17. *Mule* 2446.—

Injected as above.

Result.—Distinct reaction.

Tested on the 23rd January with the three strains of mule virus, injected separately. No reaction.

18. *Mule* 2462.—

Injected as above.

Result.—Distinct reaction.

Tested on the 23rd January by an injection of 2 c.c. virus of the three strains of horses separately. Distinct reaction.

19. *Mule* 2459.—

Injected as above.

Result.—Slight but distinct reaction.

Tested on the 23rd January by an injection of 2 c.c. virus OTB horse 2418. No reaction.

20. *Mule* 2465.—

Injected as above.

Result.—Distinct reaction.

Tested on the 23rd January by injection of 2 c.c. virus OTBLPW of horse 2406. Slight but distinct reaction.

Conclusion.—The immunity obtained from the injection of the three strains of horse virus protected against a subsequent inoculation of mule virus of the same strain against which they were immunised, but the test with horse virus of the same strain produced reactions.

11*b*. Virus and serum as above, but dose of serum 300 c.c.

21. *Mule* 2452.—
Injected on the 5th December with 300 c.c. serum and 2 c.c. virus mixture of horse virus O-T-B.
Result.—Distinct reaction.
Tested on the 23rd January with 2 c.c. virus of same mixture. Reaction, and death resulted on the 11th day from piroplasmosis.

22. *Mule* 2459.—
Injected as above.
Result.—Hardly any reaction.
Tested on the 23rd January by separate injections of the three strains of horse virus. Slight but distinct reaction.

23. *Mule* 2451.—
Injected as above.
Result.—Slight reaction.
Tested on the 23rd January by an injection of 2 c.c. virus OTB horse 2418; a distinct and typical reaction ensued.

24. *Mule* 2453.—
Injected as above.
Result.—Slight reaction.
Tested on the 23rd January with 2 c.c. virus OTBLPW horse 2406. Distinct reaction.

25. *Mule* 2458.—
Injected as above.
Result.—Distinct reaction.
Tested on the 4th January with virus horse 2407 Ord. strain; reaction. Retested on the 23rd January with 2 c.c. virus horse 2359 Bulawayo strain; no reaction.

26. *Mule* 2460.—
Injected as above.
Result.—Distinct reaction.
Tested on the 23rd January with 2 c.c. virus horse 1869 Tzaneen strain; retarded reaction, not typical. Retested on the 23rd January with 2 c.c. virus horse 2407 Ord. strain; doubtful reaction.

27. *Mule* 2457. —
Injected as above.
Result.—Distinct reaction.
Tested on the 3rd January with 2 c.c. virus horse 1959 Bulawayo strain; retarded reaction, not typical for horse-sickness. Retested on the 23rd January with 2 c.c. virus horse 1869 Tzaneen strain; no reaction.

Result.—The immunisation with the three strains of horse virus did not protect against a subsequent inoculation of a mixture of the same virus, but protected when injected separately. It protected against virus OTB and OTBLPW, but in both instances reactions were noted. It protected against the constituents of the virus separately injected, although some doubtful reactions were noted.

Résumé of Results.—Of 14 animals immunised with a mixture of the three strains of mule virus two died of horse-sickness when tested with horse virus of the same strains;

some showed distinct and some showed doubtful reactions when injected with constituents of the same mixture. Of 13 animals immunised with a mixture of the three strains of horse virus, one died when tested with the same mixture. There were also distinct and doubtful reactions noted after injection of the mixture and constituents of the various kinds of virus.

Conclusion.—The immunisation by means of serum of mules and horses adequate to the three strains and the subcutaneous and simultaneous inoculation of corresponding virus protected, in the majority of cases, against a subsequent inoculation of the mixture or constituents of the said virus, but not completely, inasmuch as three deaths occurred and many reactions were noted. The death was due to the injection of the three strains of virus derived from horses. It follows, therefore, that the virus of horses of the same strain is most virulent. The practical conclusion is that the immunisation against the three strains in the way indicated, viz., by mixing adequate sera and injecting the same against adequate virus mixture, does not produce sufficient immunity.

Experiment No. 4.—Serum mixture of mules (hyperimmunised with Ord. virus), of horses (hyperimmunised with Tzaneen virus), of horses (hyperimmunised with Bulawayo virus), injected subcutaneously and simultaneously with adequate virus derived from horses and mules.

(*a*) Dose of serum 300 c.c.; virus mixture of horse 2407 (Ord. strain), of horse 1869 (Tzaneen strain), of horse 2359 Bulawayo strain), 3 c.c., injected subcutaneously and simultaneously.

1. *Mule* 2484.—
   Injected on the 19th December, 1906, with 300 c.c. serum of above mixture and 3 c.c. horse virus O-T-B.
   Result.—Distinct and typical reaction.
   Tested on the 23rd January with 2 c.c. virus of same mixture. No reaction.
2. *Mule* 2485.—
   Injected as above.
   Result.—Reaction, with symptoms of dikkop, and the animal died on the 12th day.
3. *Mule* 2486.—
   Injected as above.
   Result.—Distinct reaction.
   Tested on the 23rd January with 2 c.c. virus of same mixture. No reaction.
4. *Mule* 2487.—
   Injected as above.
   Result.—Distinct reaction.
   Tested on the 23rd January with 2 c.c. virus of same mixture. No reaction.
5. *Mule* 2488.—
   Injected as above.
   Result.—Distinct reaction.
   Tested on the 23rd January with same mixture. No reaction.

Conclusion.—The immunity given to five mules by a mixture of three strains of horse virus injected in the dose of 3 c.c. protected against a subsequent inoculation of virus of the same strain.

(*b*) Dose of serum 300 c.c. virus mixture of mule 2287 (Ord. strain), of mule 2268 (Tzaneen strain), and mule 1954 (Bulawayo strain).

6. *Mule* 2489.—
   Injected on the 19th December, as indicated above.
   Result.—Distinct and typical reaction.
   Tested on the 23rd January with same mixture; a slight reaction.
7. *Mule* 2490.—
   Injected as above.
   Result.—Distinct reaction.
   Tested on the 23rd January with 2 c.c. of same mixture; a severe and high reaction.
8. *Mule* 2491.—
   Injected as above.
   Result.—Reaction.
   Tested on the 23rd January with same mixture of virus; no reaction.
9. *Mule* 2492.—
   Injected as above.
   Result.—Slight and typical reaction.
   Tested on the 23rd January with same mixture; typical reaction and signs of dikkop.
10. *Mule* 2493.—
    Injected as above.
    Result.—Typical reaction.
    Tested on 23rd January with same mixture; slight reaction.

Result.—The five mules immunised by the mixture of the three strains protected against a subsequent inoculation of the same three strains, but reactions were noted in four cases, and in one with signs of dikkop, showing that the protection was not complete.

Résumé.—The immunity obtained by the inoculation of the three strains of horse virus protects better against itself than the immunity obtained from the three strains of mule virus protects against the same three strains of mule virus.

Conclusion.—Immunity obtained from the three strains of horse virus is stronger than the immunity obtained from the three strains of mule virus.

Experiment No. 5.—Serum mixture of horses immunised with virus O, of horses immunised with virus T, and of horses immunised with virus B, injected in the dose of 200 c.c. and the corresponding virus 3 c.c. subcutaneously and simultaneously.

(*a*) Virus mixture of mules 2287 O, 2268 T, and 1954 B, 24th December, 1906.

1. *Mule* 2500.—
   Injected with 200 c.c. serum and 3 c.c. virus, as indicated above.
   Result.—Slight but distinct reaction.

Tested on the 23rd January with 2 c.c. virus same mixture; slight reaction.

2. *Mule* 2501.—
   Injected as above.
   Result.—Distinct reaction, and signs of dikkop on the 15th day.
   Tested on the 23rd January by the same virus, injected separately. No reaction.
3. *Mule* 2502.—
   Injected as above.
   Result.—Distinct reaction, with lesions of dikkop on the 16th day.
   Tested on the 23rd January with 2 c.c. virus of same mixture. Slight reaction.
   Conclusion.—The reduction of the dose of serum to 200 c.c. caused a more distinct immunisation reaction, which protected against the subsequent inoculation of the same virus, but still accompanied with a slight reaction.

(*b*) Virus mixture of horses 2407 O, 1869 T, and 2359 B.

4. *Mule* 2503.—
   Injected on the 24th December with 200 c.c. serum mixture and 3 c.c. virus mixture.
   Result.—Distinct reaction.
   Tested on the 23rd January with 2 c.c. virus of same mixture. Doubtful reaction.
5. *Mule* 2504.—
   Injected as above.
   Result.—Distinct reaction.
   Tested on the 23rd January with same virus mixture: slight reaction.
6. *Mule* 2505.—
   Injected as above.
   Result.—Distinct reaction, with signs of dikkop. The animal died on the 24th day after inoculation, with lesions of horse-sickness.
   Result.—Of three animals treated in the way indicated above one died as a result of the inoculation, and two reacted to the inoculation, and when tested on their immunity they proved refractory. There was a slight reaction, but somewhat doubtful as to its nature.
   Conclusion.—The reduction of the dose of serum to 200 c.c. permits a more severe immunisation reaction. The immunity obtained is the same as that obtained with a stronger dose of serum.

Experiment No. 6.—With serum mixture of mules hyperimmunised with O virus, of horses hyperimmunised with O virus, of horses with T virus, and horses with B virus, injected subcutaneously and simultaneously with 2 c.c. virus OTB horse 2418 (a horse injected with the three strains of virus.)

1. *Mule* 2506.—
   Injected on the 24th December, 1906, with 300 c.c. serum of above mixture and 2 c.c. virus OTB.
   Result.—Slight reaction.

Tested on the 23rd January by separate injections of 2 c.c. virus of horses 2407, 1869 and 2359. Slight reaction, not quite typical for horse-sickness.

2. *Mule* 2507.—

Injected as above.

Result.—Slight reaction.

Tested on the 23rd January with a mixture of the three-horse vira. There was a slight reaction.

Result.—The immunity obtained through the injection of the virus OTB protected against a subsequent inoculation of the constituents of the same virus when injected either separately or mixed. A slight reaction was noticed after the test.

Conclusion.—The immunity obtained from OTB virus protected against its constituents.

Experiment No. 7.—Serum mixture of horses O-T-B. Dose of serum 300 c.c.

(*a*) Virus 2418 OTB (*vide* previous Experiment).

1. *Mule* 2513.—

Injected on the 12th January, 1907, with 300 c.c. serum of above mixture and 2 c.c. virus 2418.

Result.—Reaction.

Tested on the 20th February with 2 c.c. virus mule 2287 Ord. strain; reaction. Retested on the 13th March with 2 c.c. virus mule 1954 Bulawayo strain; no reaction.

2. *Mule* 2514.—

Injected as above.

Result.—Distinct reaction with signs of dikkop on the 9th day.

Tested on the 21st February with 2 c.c. virus mule 2268 Tzaneen; typical reaction with dikkop on the 10th day. Retested on the 13th March with 2 c.c. virus mule 1954 Bulawayo; again a typical reaction.

3. *Mule* 2515.—

Injected as above, and died on the 12th day from horse-sickness.

4. *Mule* 2516.—

Injected as above.

Result.—Typical reaction.

Tested on the 21st February with 2 c.c. virus mule 1954 Bulawayo; typical reaction. Retested on the 13th March with virus 2268 Tzaneen; no reaction.

5. *Mule* 2517.—

Injected as above.

Result.—Reaction, and death from dikkop on the 13th day.

6. *Mule* 2518.—

Injected as above.

Result.—Typical reaction.

Tested on the 21st February with 2 c.c. virus mule 2287 Ord., horse 1869 Tzaneen, and mule 1954 Bulawayo; typical and distinct reaction. Retested on the 13th March with 2 c.c. virus 2287 Ord. strain; no reaction.

Conclusion.—Of six mules immunised with OTB virus 2418 and serum mixture of horses, two died. The remainder

obtained an immunity, which was, however, not complete, inasmuch as the subsequent inoculation of the various strains again produced reactions, mule 2514 even developing dikkop for a second time.

(*b*) Virus 2406 OTBLPW [*vide* Experiment No. 3 (11)].

7. *Mule* 2519.—
   Injected on the 12th January, 1907, with 300 c.c. serum and 2 c.c. virus 2406.
   Result.—Reaction.
   Tested on the 21st February with 2 c.c. virus 2287 Ord.; reaction. Retested on the 13th March with 2 c.c. virus 2268 Tzaneen; no reaction.

8. *Mule* 2520.—
   Injected as above.
   Result.—Reaction.
   Tested on the 21st February with 2 c.c. virus 2268 Tzaneen; slight reaction. Retested on the 13th March with 2 c.c. virus 1954 Bulawayo; no reaction.

9. *Mule* 2521.—
   Injected as above.
   Result.—Died of horse-sickness on the 11th day.

10. *Mule* 2522.—
    Injected as above.
    Result.—Reaction.
    Tested on the 21st February with 2 c.c. virus 1954 Bulawayo; reaction, lesions of dikkop; animal died on the 12th day, and on post-mortem examination showed lesions of horse-sickness and piroplasmosis.

11. *Mule* 2523.—
    Injected as above.
    Result.—Reaction and dikkop on the 13th day.
    Tested on the 21st February with 2 c.c. virus 2287 O, 2268 T, and 1954 B; reaction. Retested on the 13th March with 2 c.c. virus 1954 B; no reaction.

12. *Mule* 2524.—
    Injected as above.
    Result.—Reaction.
    Tested on the 21st February with 2 c.c. virus OTB 2418; slight reaction. Retested on the 13th March with 2287 Ordinary; no reaction.

Result.—Of six animals immunised with virus OTBLPW one died due to immunisation. When tested, one died with lesions of dikkop, and all showed reactions when tested with one of the constituents of the virus.

Conclusion.—The immunisation with a serum mixture composed of serum of horses hyperimmunised O, hyperimmunised T, and hyperimmunised B, and of virus of a polyvalent nature containing the constituents adequate to the serum, does not protect completely against the same constituents when injected separately or mixed. It therefore cannot be expected that the polyvalent immunity will be obtained in the way indicated. This fact is probably due

to an inadequate fitting of the serum mixture to the polyvalent virus whereby a surplus of one or the other serum occurs, overcompensating the adequate strain of the polyvalent virus, so that it does not leave any impression on the system of the animal, and therefore causes no immunity.

Experiment No. 8.—With serum OTB, viz., of horses hyperimmunised with virus origin 2418 OTB.

1. *Mule* 2581.—
   Injected on the 25th January, 1907, with 300 c.c. serum OTB and 2 c.c. virus 2418 simultaneously and subcutaneously.
   Result.—Distinct reaction.
   Tested on the 28th March with 2 c.c. virus 1954 Bulawayo; a slight but retarded reaction. Retested on the 23rd April with 2 c.c. virus 2628 Ordinary; no reaction. Retested on the 7th May with 2 c.c. virus mixture O-T-B of horse 2709, horse 2148, and horse 2168; distinct reaction.
2. *Mule* 2588.—
   Injected as above.
   Result.—Slight reaction.
   Tested on the 4th April with 2 c.c. virus 1954 Bulawayo; no reaction. Retested on the 23rd April with 2 c.c. virus horse 2709 O; no reaction. Retested on the 7th May with virus mixture O-T-B of horses 2709, 2148, and 2168; slight reaction.
3. *Mule* 2589.—
   Injected as above.
   Result.—Hardly any reaction.
   Tested on the 20th March with 2 c.c. virus 2418 OTB; no reaction. Retested on the 4th April with virus mule 2628 Ordinary, mule 2268 Tzaneen, and mule 1954 Bulawayo, injected separately; no reaction. Retested on the 23rd April with virus horses 2709, 2148, and 2298, viz., O-T-B separately; a slight atypical reaction.
4. *Mule* 2590.—
   Injected as above.
   Result.—Very slight reaction.
   Tested on the 6th March with 2 c.c. virus 2418 OTB; no reaction. Retested on the 28th March with 2 c.c. virus 2287 Ordinary; no reaction. Retested on the 23rd April with 2 c.c. virus 2298 Bulawayo; an atypical reaction. Retested on the 7th May by an injection of virus O 2709, virus T 2148, and virus B 2168; no reaction.
5. *Mule* 2591.—
   Injected as above.
   Result.—Reaction.
   Tested on the 28th March with virus of mule 2268 Tzaneen; no reaction. Retested on the 23rd April with 2 c.c. virus horse 2148 Tzaneen; reaction. Retested on the 7th May with a mixture of virus O-T-B horses 2709, 2148, and 2168; no reaction.

Conclusion.—The immunisation with serum OTB and corresponding virus OTB protected against a subsequent inoculation of any constituent of the virus separately or collectively injected. In the case of 2581, however, there was a distinct reaction to the injection of horse virus mixture. In the other instances slight and atypical reactions were noted, and it may be doubtful whether they are due to the test injection.

Experiment No. 9.—With serum OTBLPW, viz., of horses hyper-immunised with virus origin horse 2406 OTBLPW.

1. *Mule* 2592.—
   Injected on the 25th January with 300 c.c. serum OTBLPW and 2 c.c. virus horse 2406 subcutaneously and simultaneously.
   Result.—Slight reaction.
   Tested on the 4th April with 2 c.c. virus mule 2268 Tzaneen strain; no reaction. Retested on the 23rd April with 2 c.c. virus horse 2148 Tzaneen; also no reaction. Retested on the 7th May with mixture of virus O-T-B of horses 2709, 2148, and 2168; no reaction.
2. *Mule* 2594.—
   Injected as above.
   Result.—Slight reaction.
   Tested on the 4th April with 2 c.c. virus of mule 2628 Ordinary; no reaction. Retested on the 23rd April with 2 c.c. virus of horse 2148 Tzaneen; no reaction. Retested on the 7th May with mixture of horse virus 2709 Ordinary strain, 2148 Tzaneen, and 2168 Bulawayo strain; no reaction.
3. *Mule* 2595.—
   Injected as above.
   Result.—Slight reaction.
   Tested on the 28th March with 2 c.c. virus of mule 2287 Ordinary strain; reaction. Retested on the 23rd April with 2 c.c. virus of 2298 Bulawayo strain; reaction. Retested on the 7th May with mixture of horse virus 2709, 2148, and 2168; reaction.
4. *Mule* 2596.—
   Injected as above.
   Result.—Slight reaction.
   Tested on the 20th March with 2 c.c. virus of 2406 OTBLPW; no reaction. Retested on the 4th April with mixture of mule 2628 O, 2268 T, and 1954 B strain; no reaction. Retested on the 23rd April with 2 c.c. horse virus of 2709, 2148 and 2298, injected separately; slight and short reaction.

   Result.—The immunisation of four mules with serum OTBLPW and corresponding virus protected against a subsequent inoculation of any constituents of this virus injected separately or collectively. The two first animals showed no reaction to the test; the third animal had a reaction after every injection, and the fourth animal showed an atypical reaction.

Tabulated Résumé of Tests of Previous Experiments.

Polyvalent Serum and Polyvalent Virus.

Mule.	Immunisation. Serum. Orig.	Immunisation. Virus. Orig.	Immunisation. Virus. Gen.	Immunisation. Result.	I. Test. Virus. Orig.	I. Test. Virus. Gen.	I. Test. Result.	II. Test. Virus. Orig.	II. Test. Virus. Gen.	II. Test. Result.	III. Test. Virus. Orig.	III. Test. Virus. Gen.	III. Test. Result.
2388	Horses O-T-B	M. O	38	R	Tzn.	1	—	Tzn.	1	?	Ord.	38	—
		M. T	1										
		H. B	4										
2381	,,	,,	,,	R	Bul.	4	?	Bul.	4	—	Tzn.	1	?
2377	,,	,,	,,	?	Ord.	38	R	Ord.	38	—	,,	1	—
2378	,,	M. O	38	R	M. O	38	R	Tzn.	1	—	Ord.	38	—
		M. T	1		M. T	1							
2385	,,	,,	,,	?	,,	,,	—	,,	12	R	,,	38	—
2386	,,	,,	,,	—	,,	,,	R	O	38	—	,,	62	—
								T	1				
2384	,,	,,	,,	R	,,	,,	—	,,	,,	RD†	—	—	—
2387	,,	,,	,,	R	,,	,,	?	Tzn.	12	R	Ord.	38	R
2380	,,	,,	,,	?	,,	,,	—	O	38	?	,,	38	?
								T	1				
2467	Mule O + Horses O-T-B	M. O	38	R	M. O	38	?	—	—	—	—	—	—
		M. T	1		M. T	1							
		M. B	1		M. B	1							
2466	,,	,,	,,	R	H. O	38	R†	—	—	—	—	—	—
					H. T	1							
					H. B	1							
2472	,,	,,	,,	R	M. O T B	,,	?	—	—	—	—	—	—
2474	,,	,,	,,	R	H. O T B	,,	R†	—	—	—	—	—	—
2468	,,	,,	,,	R	OTB	5	—	—	—	—	—	—	—
2471	,,	,,	,,	R	,,	5	R	—	—	—	—	—	—
2475	,,	,,	,,	R	—	—	—	—	—	—	—	—	—
2473	,,	,,	,,	R	M. O	38	R	—	—	—	—	—	—
					M. T	1							
					M. B	1							
2461	,,	,,	,,	R	,,	,,	—	—	—	—	—	—	—
2464	,,	,,	,,	R	OTB	5	—	—	—	—	—	—	—
2470	,,	,,	,,	R	OTB LPW	1	—	—	—	—	—	—	—
2450	,,	,,	,,	R	Ord.	38	R	Bul.	1	—	—	—	—
2448	,,	,,	,,	R	Tzn.	1	?	Ord.	38	R	—	—	—
2469	,,	,,	,,	R	Bul.	1	R	Tzn.	1	?	—	—	—
2449	,,	H. O	38	R	M. O	38	?	—	—	—	—	—	—
		H. T	1		M. T	1							
		H. B	3		M. B	1							
2447	,,	,,	,,	R	H. O	38	R	—	—	—	—	—	—
					H. T	1							
					H. B	3							

R—Reaction. ?—Doubtful. R†—Reaction and died.
RD†—Reaction with Dikkop and died.

TABULATED RÉSUMÉ OF TESTS, ETC.—(*Continued*).

POLYVALENT SERUM AND POLYVALENT VIRUS.

Immunisation.					I. Test.			II. Test.			III. Test.		
Mule.	Serum.	Virus.		Result.	Virus.		Result.	Virus.		Result.	Virus.		Result.
	Orig.	Orig.	Gen.		Orig.	Gen.		Orig.	Gen.		Orig.	Gen.	
2446	Mule O + Horses O-T-B	H. O 38; T 1; B 3		R	M. O 38; T 1; B 1		—	—	—	—	—	—	—
2462	,,	,,	,,	R	H. O 38; T 1; B 1		R	—	—	—	—	—	—
2459	,,	,,	,,	R	OTB	5		—	—	—	—	—	—
2465	,,	,,	,,	R	OTB LPW	1	R	—	—	—	—	—	—
2452	,,	,,	38; 1; 1	R	H. O 38; T 1; B 1		R†	—	—	—	—	—	—
2459	,,	,,	,,	?	,,	,,	R	—	—	—	—	—	—
2451	,,	,,	,,	R	OTB	5	R	—	—	—	—	—	—
2453	,,	,,	,,	R	OTB LPW	1	R	—	—	—	—	—	—
2458	,,	,,	,,	R	Ord.	38	R	Bul.	1	—	—	—	—
2460	,,	,,	,,	R	Tzn.	1	?	Ord.	38	?	—	—	—
2457	,,	,,	,,	R	Bul.	3	?	Tzn.	1	—	—	—	—
2484	Mule O + Horses T B	H. O 38; T 1; B 1		R	H. O 38; T 1; B 1		—	—	—	—	—	—	—
2485	,,	,,	,,	RD†	—	—	—	—	—	—	—	—	—
2486	,,	,,	,,	R	H. O 38; T 1; B 1		—	—	—	—	—	—	—
2487	,,	,,	,,	R	,,	,,	—	—	—	—	—	—	—
2488	,,	,,	,,	R	,,	,,	—	—	—	—	—	—	—
2489	,,	M. O 38; T 1; B 1		R	M. O 38; T 1; B 1		R	—	—	—	—	—	—
2490	,,	,,	,,	R	,,	,,	R	—	—	—	—	—	—
2491	,,	,,	,,	R	,,	,,	—	—	—	—	—	—	—
2492	,,	,,	,,	R	,,	,,	RD	—	—	—	—	—	—
2493	,,	,,	,,	R	,,	,,	R	—	—	—	—	—	—
2500	Horses O-T-B	M. O 38; T 1; B 1		R	M. O 38; T 1; B 1		R	—	—	—	—	—	—
2501	,,	,,	,,	RD	,,	,,	—	—	—	—	—	—	—
2502	,,	,,	,,	RD	,,	,,	R	—	—	—	—	—	—
2503	,,	H. O T B	,,	R	H O T B	,,	?	—	—	—	—	—	—
2504	,,	,,	,,	R	,,	,,	R	—	—	—	—	—	—
2505	,,	,,	,,	RD†	—	—	—	—	—	—	—	—	—

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop. R†—Reaction and died.
RD†—Reaction with Dikkop and died.

Tabulated Resumé of Tests, etc.—(*Continued*).

Polyvalent Serum and Polyvalent Virus.

Mule.	Immunisation. Serum. Orig.	Immunisation. Virus. Orig.	Immunisation. Virus. Gen.	Immunisation. Result.	I. Test. Virus. Orig.	I. Test. Virus. Gen.	I. Test. Result.	II. Test. Virus. Orig.	II. Test. Virus. Gen.	II. Test. Result.	III. Test. Virus. Orig.	III. Test. Virus. Gen.	III. Test. Result.
2506	Mule O + Horses O-T-B	OTB	5	R	Il. O	38	?	—	—	—	—	—	—
					Il. T	1							
					Il. B	1							
2507	,,	,,	5	R	,,	,,	R	—	—	—	—	—	—
2513	Horses O-T-B	,,	5	R	Ord.	38	R	Bul.	1	—	—	—	—
2514	,,	,,	5	RD	Tzn.	1	RD	Bul.	1	R	—	—	—
2515	,,	,,	5	R†	—	—	—	—	—	—	—	—	—
2516	,,	,,	5	R	Bul.	1	R	Tzn.	1	—	—	—	—
2517	,,	,,	5	RD†	—	—	—	—	—	—	—	—	—
2518	,,	,,	5	R	M. O	38	R	Ord.	38	—	—	—	—
					H. T	1							
					M. B	1							
2519	,,	OTB LPW	1	R	Ord.	38	R	Tzn.	1	—	—	—	—
2520	,,	,,	1	R	Tzn.	1	R	Bul.	1	—	—	—	—
2521	,,	,,	1	R†	—	—	—	—	—	—	—	—	—
2522	,,	,,	1	R	Bul.	1	RD†	—	—	—	—	—	—
2523	,,	,,	1	RD	M. O	38	R	Bul.	1	—	—	—	—
					M. T	1							
					M. B	1							
2524	,,	,,	1	R	OTB	5	R	Ord.	38	—	—	—	—
2581	OTB	OTB	5	R	Bul.	1	R	Ord.	38	—	H. O	70	R
											H. T	13	
											H. B	10	
2588	,,	,,	5	R	,,	1	—	Ord.	70	—	,,	,,	R
2589	,,	,,	5	?	OTB	5	—	M. O	38	—	,,	70	?
								M. T	1			13	
								M. B	1			11	
2590	,,	,,	5	R	,,	5	—	Ord.	38	—	Bul.	11	?
											H. O	70	—
									4th	test	H. T	13	
											H. B	10	
2591	,,	,,	5	R	Tzn.	1	—	Tzn.	13	R	,,	,,	—
2592	OTB LPW	OTB LPW	1	R	,,	1	—	,,	13	—	H. O	70	—
											H. T	13	
											H. B	10	
2594	,,	,,	1	R	Ord.	38	—	,,	13	—	,,	,,	—
2595	,,	,,	1	R	,,	38	R	Bul.	11	R	,,	,,	R
2596	,,	,,	1	R	OTB LPW	1	—	M. O	38	—	,,	70	R
								M. T	1			13	
								M. B	1			11	

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop. R†—Reaction and died.
RD†—Reaction with Dikkop and died.

Extracts from above Tabulated Résumé.

Deaths from Immunisation.

A.—3 mules, immunised with serum horses O-T-B and virus { M. [O 38 gen. / T 1 gen.] / H. B 1 gen. } : .. .. No deaths.

6 mules, immunised with same serum as above and virus M. [O 38 gen. / T 1 gen.] : .. .. No deaths.

3 mules, immunised with same serum as above and virus M. [O 38 gen. / T 1 gen. / B 1 gen.] : .. .. No deaths.

3 mules, immunised with same serum as above and virus H. [O 38 gen. / T 1 gen. / B 1 gen.] .. .. 1 death.

6 mules, immunised with serum as above and virus OTB, 5th generation .. .. 2 deaths.

6 mules, immunised with serum as above and virus OTBLPW, 1st generation .. .. 1 death.

B.—5 mules, immunised with serum [M.O / H.T / H.B] and virus H. [O 38 gen. / T 1 gen. / B 1 gen.] : .. .. 1 death.

5 mules, immunised with serum as above and virus M. [O 38 gen. / T 1 gen. / B 1 gen.] : .. .. No deaths.

C.—14 mules, immunised with serum [M.O / H.O / H.T / H.B] and virus M. [O 38 gen. / T 1 gen. / B 1 gen.] : .. .. No deaths.

13 mules, immunised with serum as above and virus H. [O 38 gen. / T 1 gen. / B 1 gen.] : .. .. No deaths.

2 mules, immunised with serum as above and virus OTB, 5th generation .. .. No deaths.

D.—5 mules, immunised with serum OTB and virus OTB, 5 gen. No deaths.

E.—4 mules, immunised with serum and virus OTBLPW, 1 gen. No deaths.

Tests.

A.—3 mules, immunised serum horse O-T-B and virus M. $\left\{\begin{matrix}O\\T\\H.\ B\end{matrix}\right\}$.

1 Mule, 1st and 2nd test with Tzaneen and 3rd test with Ord., 38th generation, had a doubtful reaction with 2nd test (Tzaneen, 1st generation).

1 Mule, 1st and 2nd tests with Bulawayo, 4th generation, and 3rd test with Tzaneen, 1st generation, had a doubtful reaction with 1st test (Bulawayo) and with 3rd test (Tzaneen).

1 Mule, 1st and 2nd tests with Ord., 38th generation, and 3rd test with Tzaneen, 1st generation, had a distinct reaction with the 1st test (Ord.)

6 Mules, immunised with serum horse O-T-B and virus mules $\left\{\begin{matrix}T\\O\end{matrix}\right\}$.

2 Mules, 1st test M. $\left\{\begin{matrix}O\ 38\ gen.\\T\ \ 1\ gen.\end{matrix}\right\}$ 2nd test with Tzaneen, 12th generation, and 3rd test with Ord., 38th generation; 1 had doubtful reaction with 1st and reactions with the 2nd and 3rd tests; 1 had reaction with the 2nd test (Tzaneen).

1 Mule, 1st test with M. $\left\{\begin{matrix}O\ 38\ gen.\\T\ \ 1\ gen.\end{matrix}\right\}$ 2nd test with Tzaneen, 1st generation, and 3rd test with Ord., 38th generation, had a reaction with the 1st test.

1 Mule, 1st and 2nd tests with M. $\left\{\begin{matrix}O\ 38\ gen.\\T\ \ 1\ gen.\end{matrix}\right\}$ and 3rd test with Ord., 62nd generation, had a reaction with the 1st test only.

1 Mule, 1st and 2nd tests with M. $\left\{\begin{matrix}O\ 38\ gen.\\T\ \ 1\ gen.\end{matrix}\right\}$ and 3rd test with Ord., 38th generation, had doubtful reactions with the 2nd and 3rd tests.

1 Mule, 1st and 2nd tests with M $\left\{\begin{matrix}O\ 38\ gen.\\T\ \ 1\ gen.\end{matrix}\right\}$ had no reaction with 1st test, but reaction, dikkop, and died on the 2nd test.

3 Mules, immunised with serum horses O-T-B and virus mules $\left\{\begin{matrix}O\\T\\B\end{matrix}\right\}$.

Tested with M. $\left\{\begin{matrix}O\ 38\ gen.\\T\ \ 1\ gen.\\B\ \ 1\ gen.\end{matrix}\right\}$; 2 reactions.

2 Mules, immunised with serum horses O-T-B and virus horse $\left\{\begin{matrix}O\\T\\B\end{matrix}\right\}$.

Tested with H. $\left\{\begin{matrix}O\ 38\ gen.\\T\ \ 1\ gen.\\B\ \ 1\ gen.\end{matrix}\right\}$; 1 distinct and 1 doubtful reaction.

4 Mules, immunised with serum horses O-T-B and virus OTB.

1 Mule, 1st test with Ord., 38th generation, gave a reaction, and 2nd test with Bulawayo, 1st generation, gave no reaction.

1 Mule, 1st test with Tzaneen, 1st generation, reaction and dikkop, and also a reaction with 2nd test Bulawayo, 1st generation.

1 Mule, 1st test Bulawayo, 1st generation, gave a reaction, and none with 2nd test, Tzaneen, 1st generation.

1 Mule, tested with { M.O 38 gen. / H.T 1 gen. / M.B 1 gen. } and showed a reaction ; 2nd test with Ord., 38th generation, no reaction.

5 Mules, immunised with serum horses O-T-B and virus OTBLPW.

1 Mule, 1st test with Ord., 38th generation, and 2nd test with Tzaneen, 1st generation, had reaction with the 1st test.

1 Mule, 1st test with Tzaneen, 1st generation, and 2nd test with Bulawayo, 1st generation, had reaction with the 1st test.

1 Mule, 1st test with M. { O 38 gen. / T 1 gen. / B 1 gen. } and 2nd test with Bulawayo, 1st generation, reacted with the 1st test.

1 Mule, 1st test with OTB, 5th generation, and 2nd test with Ord., 38th generation, also reacted on the 1st test.

1 Mule, tested with Bulawayo, 1st generation, reacted and died.

B.—4 Mules, immunised with serum { M.O / H.T / H.B } and virus horse { O / T / B } and tested with the same virus, gave no reaction.

5 Mules, immunised with serum as above and virus mules { O / T / B } and tested with the same virus, gave 3 reactions and 1 reaction and dikkop.

C.—14 Mules, immunised with serum { M.O / H.O / H.T / H.B } and virus mules { O / T / B }.

4 Mules, tested with same virus ; 1 reaction and 2 doubtful reactions.

2 Mules, tested with virus horse { O 38 gen. / T 1 gen. / B 1 gen. }, reacted and died.

3 Mules, tested with OTB, 5th generation; only 1 gave a reaction.

1 Mule, tested with OTBLPW, 1st generation, gave no reaction.

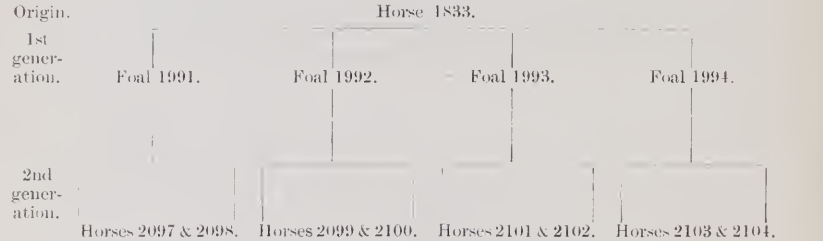
1 Mule, 1st test with Ord., 38th generation, and 2nd test with Bulawayo, 1st generation, reacted with 1st test.

"H."—CONTINUATION OF EXPERIMENTS FOR INOCULATION AGAINST EQUINE PIROPLASMOSIS.

In my last Annual Report a number of experiments were enumerated showing (1) that the inoculation of mules with immune mule blood can be performed with a large prospect of success; (2) that a certain amount of risk is attached to the inoculation of donkeys with immune mule blood; and (3) that the inoculation of horses with immune donkey blood may be followed by disastrous results. During the past year these experiments have been continued on a somewhat different line, and based on the observation made in connection with redwater, namely, that the inoculation of cattle with blood of a calf immune against this disease is not so frequently followed by strong reactions and mortality as when the blood is derived from a full-grown beast.

Accordingly, I decided to utilise the blood (*a*) from young immune weaned horse foals, and (*b*) from immune donkey foals which are still suckling. The experiments were classified according to the origin of the blood, namely, (1) from an immune horse, (2) from an immune mule, and (3) from an immune donkey. At the commencement of each of the three classes of experiments I attach the genealogical table of the animals whose blood was utilised for the inoculation.

1.—Inoculation against Equine Piroplasmosis by means of Horse Foal Blood—Origin Horse Blood.



1.—Inoculation by means of Horse Foal Blood—Origin Horse Blood.

Horse 1833.—Argentine, aged (compare Annual Report, 1905-6 page 94). This horse had been injected with blood of mule 589—immune against piroplasma equi—and showed two reactions accompanied by piroplasma equi, but in rare numbers.

Transvaal Foals injected with immune horse blood.—Transvaal foals Nos. 1991, 1992, 1993 and 1994, all six months, and obtained from the S.A.C., were injected on the 25th May, 1906, subcutaneously with ·5 c.c. defibrinated fresh blood of horse 1833.

Experiment 1.

1. *Foal* 1991.— *First Generation.*

Injected as above.

Irregular reaction, commencing on the 8th day and continuing for 18 days. On the 27th day—the morning temperature being 101.8—a sharp reaction was noticed for five days, the

maximum temperature being 105 F. on the evening of the 28th, and falling to 101 at the conclusion. The ring form of piroplasma equi was noticed in rare numbers for the first time on the 12th day; temperature 99.6 in the morning and rising to 101 in the evening. Piroplasma equi—chiefly spherical form—present in fairly large numbers the following day, causing the temperature to rise from 101.6 in the morning to 103 in the evening. One rosette also present on this date. Further rise to 103.6 in the evening of the next day, but no piroplasms observed. Spherical ring and irregular forms in very small numbers noticed at intervals until the 31st day, the evening temperature reading 103 F. Mucous membrane injected on the 22nd day, but were clearer next morning. No further piroplasms observed; the temperature remained normal, and, accordingly, the examinations were discontinued.

The animal recovered.

2. *Foal* 1992.—

Injected as above.

Temperature reaction commenced on the 9th day and reached 106 F. in the morning of the 20th day; it now slowly fell and recorded 98.4 F. on the morning of the 33rd day. A second reaction ensued, reaching the maximum of 104 F. on the mornings of the 38th and 39th days, but gradually fell and remained normal from the 50th day.

No piroplasms observed until the 20th day, and these were in extremely rare numbers—the temperature at that time recording the maximum of 106. The ring forms were noticed in fair numbers on the 22nd day, with a temperature record of 105 F.; no further piroplasms were observed until the commencement of the second reaction, when the ring forms were found in rare numbers on the 39th day—the temperature showed 101 and 102.8 in the morning and evening of this date respectively.

Mucous membranes were slightly injected on the 19th day, and a slight coryza was present; the mucous membranes were icteric and echymosed on the 22nd day, but attained a normal state the following morning, although the animal's appearance was not good.

No further piroplasms were observed, and the examinations were accordingly discontinued.

3. *Foal* 1993.—

Injected as above.

A short reaction on the 7th, 8th, 9th and 10th days, rising from 99 F. The curve now assumed a regular reaction, lasting until the 31st day, and touching the maximum of 104.2 F. on the 20th day. This was followed by a second rise of a fairly regular nature until the morning of the 46th day, when the temperature recorded 99.4 F., but was immediately followed by a sharp rise to 106 in the evening of the 48th day. Temperature now returned to normal.

Piroplasms in very rare numbers noted for the first time on the 8th and 9th days, coinciding with the rise of temperature to 103. Examinations gave negative results until four days later, when one piroplasma equi and one rosette were present. Another piroplasm seen on the 16th day accompanied with the

lesions of a slight poikilocytosis. Spherical ring, leaf, and irregular forms occasionally noted in very rare numbers from the 19th to 33rd days, but further examinations gave negative results. Mucous membranes injected and icteric on the 22nd days. On the 26th day a rather profuse coryza was present, but no further lesions observed.

Examination discontinued.

Recovered.

4. *Foal* 1994.—

Injected as above.

Reaction of a regular nature lasted from the 6th day, until the evening of the 16th day, when a sharp rise occurred to 104.8 on the following evening, and 105 F. on the evenings of the 19th and 20th days. It decreased from this date, and assumed a regular but slight reaction between 100 and 102 F. for the next 25 days.

The ring form in rare numbers observed for the first time on the 9th day, accompanied with the appearance of a few megaloblasts. All examinations negative until the 18th day, when a slight coryza was present, and the mucous membranes were injected. Piroplasms very scarce the following day, and on the 20th day the mucous membranes were observed to be slightly yellow. Mucous membranes slightly jaundiced on the 22nd day, but clearer the following morning. Piroplasms were still absent. The lesions of a slight poikilocytosis observed on the 27th day, and two days later the ring form was present in very rare numbers. These ring forms increased in number the following day, but coinciding with the fall in temperature from 102 F. to 99.4, the latter being obtained on the 33rd day. The number of piroplasms decreased, and on the 34th day only one rosette was present.

Further examinations proved negative, and consequently were discontinued from the 37th day.

Recovered.

Results of four Transvaal horse foals injected with blood of a horse immune against piroplasma equi all showed a reaction due to this inoculation, accompanied with piroplasms, and none died.

Injections of Argentine Horses with Blood of Transvaal Horse Foals.

Experiment No. 2.

Second Generation.

(a) *Injections with blood of horse foal* 1991.

1. *Horse* 2097.—Mare about four years old, directly imported from the Argentine.

Injected on the 9th July with 5 c.c. fresh defibrinated blood from foal 1991.

Reaction commenced on the 10th day, rising to 106 in the morning of the 12th day. A fairly sharp drop from this reading to 99.4 on the morning of the 17th day, was followed by a regular reaction between 101 and 105 F., which lasted until death on the 48th day. Coinciding with the rise to 106 F. numerous ring forms were present, and remained in fair numbers for the next

six days, but two days later—19th day after injection—they were recorded as very scarce.

On the 14th day—two days after the maximum of 106 F.—the mucous membranes were yellow, and hindquarters weak. On the 16th day the mucous membranes were still yellow, and also echymosed, remaining yellow until the 25th day. Piroplasms again present in rare numbers on the 26th day, and a few points were observed three days later, increasing on the 31st day, and remaining in fair numbers until the 46th day.

Piroplasms in scarce numbers noted for the last time on the 36th day, and twelve days later the animal died from broncho-pneumonia, complicated with pregnancy.

Post-mortem Examination.—

Condition:—Good. Uterus pregnant; vulva swollen; blood of a normal colour.

Lungs:—Right middle lobe contained a patch of hepatisation with mucus in the bronchi; in the left middle lobe was an abscess the size of a walnut; right posterior lobe shows white thrombi in the veins.

Heart:—Ventricles normal but rather pale.

Spleen:—Normal.

Liver:—Normal.

Stomach:—Normal.

Kidneys:—Pale; capsula not easily stripped off.

Intestines:—Mucous membranes pale.

2. *Horse* 2098.—Three-year-old mare, directly imported from Argentine.

Injected on the 9th July, 1906, with 5 c.c. fresh defibrinated blood of foal 1991.

Irregular reaction from date of injection. A typical reaction commenced on the 13th day until the 24th day, and reaching the maximum of 105.8 on the 19th day. On the 28th day another sharp rise ensued, lasting for four days, and followed by a slight reaction between 99 and 103 until the 56th day.

Examinations.—Two piroplasms seen for the first time on the 12th day, and two days later piroplasma equi, and rosettes were present in fair numbers. The hindquarters of the animal were noticed to be slightly weak during the first rise, but no piroplasma seen at the time of the maximum temperature. On the 21st day—temperature being 103 in the morning and 104.8 F. in the evening—the mucous membranes were pale, and two points were present.

On the 25th day—at the conclusion of the primary rise—the animal's legs were weak, but no piroplasms were observed until the day after the second rise. Piroplasms and points were present in fair numbers for the next eight days, but on the 40th day only a few points were visible. These points were present in rare numbers on the 45th, 47th, 49th and 51st days, and on this latter date the urine was tested, but no traces of albumen found. One piroplasm was found on the 56th day, and two days later another piroplasm, also a few points. All further examinations proved negative, and were accordingly discontinued.

The animal recovered.

(b) *Injections with blood of foal* 1992.

3. *Horse* 2099.—Mare. 3½ years old, and directly imported from the Argentine.

Injected on the 9th July, 1906, subcutaneously with 5 c.c. fresh blood of foal 1992.

Reaction commenced on the 14th day, rising to a maximum of 105.4 on the 21st day, when the mucous membranes were noticed to be pale. Temperature gradually fell for the next six days, but suddenly dropped to a sub-normal record of 96.4 on the 28th day. The animal, however, rallied, and the temperature fluctuated between normal and 103.4 for the next eight days. Death occurred on the 15th August—37 days after injection—from piroplasmosis, complicated with pregnancy.

Blood Examinations.—Piroplasms noticed in very rare numbers for the first time on the 12th day. A few rosette forms noticed a few days later; piroplasms increased on the 15th and 16th days, but only two were observed on the 17th day. A slight poikilocytosis recorded on the 19th day. Examinations proved negative during the rise of temperature to 105.4, and were not observed until the 29th day, just after the animal had rallied from 96.4 F., when piroplasms and points were observed in fair numbers, and on the day of death were very numerous.

Post-mortem Examination.—

Condition:—Fair; mucous membranes blanched; uterus pregnant.
Lungs:—Slightly œdematous.
Heart:—About an ounce of clear fluid in pericard.
Spleen:—Slightly swollen.
Liver:—Slightly swollen, but not icteric.
Stomach:—A few hæmorrhages on mucous membranes.
Kidneys:—Swollen; capsulas adherent.
Intestines:—A few punctate hæmorrhages in cæcum.

4. *Horse* 2100.—Mare, two years old, and directly imported from the Argentine.

Injected on the 9th July subcutaneously with 5 c.c. fresh defibrinated blood of foal 1992.

Temperature fluctuated directly after injection, from 98 F. in the morning to 102 in the evening. A regular reaction commenced on the 11th day, rising to 105 F. three days later, and accompanied on this date with a yellowish appearance of mucous membranes. Slight fall noticed for the next three days, and, in the morning of the 17th day—temperature being 101—the mucous membranes were slightly yellow; the hindquarters were weak. In the afternoon of the same day the animal died, the cause of death being recorded as gangrenous pneumonia.

Examinations.—Piroplasms were present for the first time on the 12th day; noticed in rare numbers the following morning, and very numerous at the time of the maximum temperature of 105 F. No piroplasms observed at death.

Post-mortem Examination.—

Condition:—Fair: mucous membranes pale: blood-stained discharge from nostrils.

Lungs:—Anterior lobe and lower edge of left lung gangrenous; almost the whole of the remainder of the left lung hepatised: ecchymoses on pleura.
Heart:—Normal.
Spleen:—Enlarged.
Stomach:—Normal.
Kidneys:—Swollen and pale.
Liver:—Normal.
Intestines:—Normal.

(c) *Injections with blood of horse foal* 1993.

5. *Horse* 2101.—Mare, three years old, and directly imported from the Argentine.

Injected subcutaneously on the 9th July, 1906, with 5 c.c. fresh blood of foal 1993.

Reaction started immediately, rising to 104 on the 7th day, but the animal died the following morning from gastro-enteritis (probably horse-sickness).

All examinations negative.

Post-mortem.—
Condition:—Poor.
Lungs:—Normal.
Heart:—Normal.
Spleen:—Normal.
Liver:—Normal.
Kidneys:—Normal.
Stomach:—Intense patchy congestion.
Intestines:—Duodenum as far as end of large colon congested; remainder normal.

6. *Horse* 2102.—Mare, three years old, directly imported from the Argentine.

Injected on the 9th July, 1906, subcutaneously with 5 c.c. fresh blood of foal 1993.

Temperature.—Reaction from the 6th day, rising to 104.6 on the 14th day, but falling to 99.8 two days later. For the next sixteen days it fluctuated between 99.6 and 105.6, on the 34th day recording 99.4, which marked the commencement of a secondary reaction, lasting for about 18 days. This secondary reaction was of a very irregular character, and fluctuated between 98.8 in the morning to 104 in the evening.

Examinations.—The ring form of piroplasma noticed for the first time on the 10th day in fair numbers, and irregular forms present the following day. Occasional points and piroplasms observed on the 15th, 16th, 17th, 18th, and 20th days, but none were seen at the time of the maximum temperature on the 23rd to 26th days. The lesions of a slight poikilocytosis were present on the 26th day, and, four days later, piroplasms and points again appeared. These were present almost daily until the 67th day, this being the last time any were present. Future examinations gave negative results, and were accordingly discontinued from the 75th day. The lesions of a slight poikilocytosis were again noticed on the 50th and 58th day after injection.

Recovered.

(d) *Injections with blood of horse foal* 1994.

7. *Horse* 2103.—Four-year-old mare, directly imported from the Argentine.

Injected on the 9th July, 1906, with 5 c.c. defibrinated fresh blood of foal 1994.

Reaction commenced on the 11th day, reaching the maximum of 105.4 on the 26th day, and dropping to 97 on the morning of the 32nd day. Recovered to 101.6 in the evening and now remained normal.

Examinations.—One piroplasm seen for the first time on the 11th day coinciding with the commencement of the reaction. Piroplasms and rosettes noticed for the next six days, and, on this latter date—the 17th day after injection—the mucous membranes were slightly yellow and the hindquarters weak. Piroplasms in rare numbers were present on the 21st day—the morning temperature being 101.4, and, in the evening, 104.1. No piroplasms seen during the time of the maximum temperature and remained absent until two days previous to the sub-normal record of 97, on which day—the 30th after the injection—two piroplasms were present and several points. A few points were present on the 37th and 40th days, and one piroplasm on the 44th day.

The animal recovered.

8. *Horse* 2104.—Three-year-old mare, and directly imported from the Argentine.

Injected on the 9th July, 1906, with 5 c.c. defibrinated blood of foal 1994.

Temperature Records.—Typical reaction started on the 11th day, rising to 103.2 in the morning of the 19th, and 106 in the evening of the 21st, but steadily dropped, and recorded 99.2 on the morning of the 29th day. It remained normal for five days, but, on the 35th day, started to rise, reaching 103.2 on the evening of the 37th day, and suddenly fell next morning.

The animal died the same afternoon.

Examinations.—Piroplasms seen for the first time on the 15th day, remaining present for four days. Mucous membranes slightly yellow on the 17th day, and in the same condition on the day of the maximum temperature of 106—the 21st day after injection—on which date one piroplasm and a few points were noted. No further piroplasms observed until the temperature dropped to normal, when, on the 30th, 31st and 32nd days, points were present in fair numbers. Coinciding with the second rise, the piroplasms increased, and, on the three days previous to death, were present in extremely large numbers.

The animal died on the 17th August from piroplasmosis, complicated with pregnancy.

Post-mortem.—

Condition:—Good; uterus pregnant. Subcutaneous tissue very icteric.

Lungs:—Pale.

Heart:—One ecchymose in left ventricle; a few petechiae (in one patch) in right ventricle.

Spleen:—Slightly swollen.

Liver:—Very slightly icteric.

Stomach:—Normal.

Intestines:—Cæcum slightly congested; fæces yellow: numerous filaria papillosa in peritoneal cavity.

Results of eight Argentine mare horses injected with blood of Transvaal horse foal (1st generation) immune against piroplasma equi. They all showed a reaction accompanied with the appearance of piroplasma equi, and five died. In two of these cases, however, the mares were in foal, and no doubt this factor had some bearing on the mortality, but, at the same time, it will be noted from the post-mortem reports that two deaths were complicated with pneumonia —probably contracted on board ship, one of these animals also being a mare heavy in foal.

2.—Inoculation against Equine Piroplasmosis by Means of Horse Foal Blood—Origin Mule Blood.

Mule 589		...	...	Origin.
Horse foal 1766		...	...	1st generation.
Horse foal 2053.	Horse foal 2054	...	...	2nd generation.
	Horse foal 2314	...	...	3rd generation.
	Horse foal 2273	...	...	4th generation.
	Horse foal 2274	...	...	5th generation.
	Horse foal 2621	...	...	6th generation.
	Horse foal 2734	...	...	7th generation.
	Horse foal 2786	...	...	8th generation.

2.—Inoculation by Means of Horse Foal Blood—Origin Mule Blood.

Mule 589.—(Compare "Further Notes on Piroplasmosis of the Horse, Mule and Donkey," Annual Report, 1904-5, Experiment "B," No. 1, page 98).

This was an Argentine mule, and had been injected with blood of three horses immune against piroplasma equi. Two reactions due to the injection and piroplasma equi present during primary reaction on the 7th day for the first time; unfortunately no microscopical examinations were made during secondary reaction.

Experiment No. 3.

First Generation.

1. *Horse Foal* 1766.—Transvaal foal, about six months old.

Injected on the 19th April, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of mule 589.

Very slight reaction from date of injection, the temperature consistently remaining between 99 and 103 for 63 days.

Examinations.—No piroplasms seen until the 22nd day, but then only in very rare numbers. They were never numerous, but were present on the 23rd, and from the 26th to 30th days. All further examinations negative, and accordingly discontinued from the 57th day.

Result.—One Transvaal horse foal injected with blood of a mule immune against piroplasma equi, passed through a slight reaction, accompanied with piroplasms.

Experiment No. 4.

1.—*Transvaal horse foals injected with blood of Transvaal horse foal* 1766, *immune against piroplasma equi.*

Second Generation.

Horse foals 2053 and 2054, both obtained from S.A.C., were injected on the 4th July, 1906, subcutaneously with 5 c.c. defibrinated blood of Transvaal horse foal 1766.

1. *Horse Foal* 2053.—Nine-month-old gelding obtained from S.A.C.

Injected as above.

Temperature.—Sharp rise from 99.4 in the morning of the 6th day to 104.2 in the evening. The regular reaction commenced on the 9th day and reached the maximum of 104 in the evening of the 20th. A second reaction now ensued, the temperature remaining between 100 and 103.6 for the next 16 days, after which it resumed a normal aspect.

Examinations.—Piroplasms in scarce numbers observed for the first time on the 15th day, and remained present for two days, one ring also being noted on the 16th day. On the day of the maximum temperature—the 20th—one piroplasm was present, and the following day points were noticed. Points again present on the 27th and 37th days; one piroplasm noted on the 25th day and two on the 38th day. All further examinations proved negative and were accordingly discontinued.

The animal recovered.

2. *Horse Foal* 2054.—A six-month-old gelding.

Injected as above.

Temperature reached 104 on the 3rd day, but dropped to normal six days later, when a regular reaction ensued, lasting for 20 days, and reaching the maximum of 103.4 on the 21st day after injection. A secondary rise noted from the 27th day, rising to 104 two days later, and remaining between 99 and 103 for 18 days.

Examinations.—Piroplasma equi in rare numbers present for the first time on the 16th and 17th days. Coinciding with the

commencement of the second reaction, one piroplasm was present on the 27th and following day. All further examinations were discontinued from the 41st day.

The animal recovered.

Results of two Transvaal horse foals injected with blood of foals immune against piroplasma equi (first generation), both showed a reaction, accompanied with piroplasms, and recovered.

2.—*Argentine horses injected with blood of Transvaal horse foal* 1766, *immune against piroplasma equi.*

3. *Horse* 2067.—Two-year-old mare, directly imported from the Argentine.

Injected on the 16th June, 1906, with 5 c.c. defibrinated fresh blood of foal 1766.

Reaction commenced on the 9th day, reaching the maximum of 105.4 in the evening of the 13th day, and returning to normal on the 23rd day. Five days later a second reaction ensued, rising from 99.8 in the morning to 106 in the evening. It gradually fell for the next two days, and remained between 101.4 and 104.2 until the 42nd day. The animal died two days later from the sequel of piroplasmosis.

Examinations.—Coinciding with the rise to 105.6 on the 13th day, piroplasms were present in large numbers, but very scarce the following day. All other examinations negative. Mucous membranes pale and yellowish, and number of corpuscles on the 14th dropped from 3,900,000 per c.m.m. to 2,100,000.

4. *Horse* 2075.—Argentine, two years old, mare, and directly imported.

Injected on the 9th July, 1906, with 5 c.c. fresh defibrinated blood of foal 1766.

Reaction commenced on the 5th day, rising to 104 in the evening of the 8th day, but fell to 99.6 in the morning of the 11th day. The animal died the same evening from syncope.

Result.—Of two Argentine mare horses injected with blood of a Transvaal foal immune against piroplasma equi (first generation), both showed a reaction, and subsequently died, one of the sequel of piroplasmosis and the other from syncope.

3.—*Argentine donkeys, injected with blood of Transvaal horse foal* 1766, *immune against piroplasma equi.*

Second Generation.

Note.—The following donkeys were all injected on the 16th June, 1906, with 5 c.c. fresh defibrinated blood of foal 1766.

5. *Donkey* 841.—Argentine, six-year-old mare.
Injected as above.
Temperature.—No distinct reaction.

6. *Donkey* 842.—Five-year-old stallion, imported from the Argentine.
Injected as above.
Temperature.—No distinct reaction.
Recovered.

7. *Donkey* 843.—Six years old; Argentine mare.
Injected as above.
Temperature.—No distinct reaction.
Recovered.

8. *Donkey* 844.—Four-year-old Argentine mare.

Injected as above.

Temperature.—No distinct reaction

9. *Donkey* 845.—Four-year-old mare, Argentine.

Injected as above.

Temperature.—No distinct reaction.

Result.—Of five Argentine donkeys injected with blood of a Transvaal foal (first generation), immune against piroplasma equi, none showed a distinct reaction, neither did any die.

Experiment No. 5.

Third Generation.

Argentine mules injected with blood of Transvaal horse foal 2053, *immune against piroplasma equi.*

Argentine mules 2315, 2320, 2321, 2322, 2324 and 2325 were injected on the 5th April, 1906, with 5 c.c. fresh defibrinated blood of Transvaal horse foal 2053.

1. *Mule* 2315.—Aged gelding.

Injected as above.

Temperature.—Reaction commenced on the 8th day, and recorded 104.8, 105 and 105 in the evenings of the 13th, 14th and 15th days respectively. It now dropped and remained normal for 11 days. On the 28th day, a second reaction ensued, reaching the maximum of 105 five days later. The temperature fell sharply and remained normal from the 39th day.

Examinations.—Piroplasms present for the first time on the 11th day, and remained in fair numbers during the height of the first reaction. A single piroplasm noticed on the 19th day, and coinciding with the second reaction; the lesions of poikilocytosis and piroplasms were present on the 33rd and 34th days. All further examinations negative.

2. *Mule* 2320.—Three-year-old Argentine gelding.

Injected as above.

Temperature.—Reaction commenced on the 11th day, rising to 103.4 in the evening, and 103.8 twenty-four hours later, but fell and remained normal from the 15th day.

Examinations.—The lesions of poikilocytosis were noticed on the 12th and 13th days, and on this latter date one point was present. Piroplasma equi noted for the first time on the 15th day and again four days later.

Recovered.

3. *Mule* 2321.—Three-and-a-half-year-old Argentine.

Injected as above.

Temperature.—Very slight reaction from the 10th day, reaching 102.4 in the evening two days later.

Examination.—Two points and the lesions of a slight poikilocytosis present on the 13th day, and two days later piroplasms in fair numbers seen for the first time. Poikilocytosis also noted on the 18th and following day.

Recovered.

4. *Mule* 2322.—Argentine gelding, two and a half years old.

Injected as above.

Temperature.—Slight reaction from the 8th day, recording the maximum temperature of 102.4 on the 16th day, but slowly fell from that date, and remained normal.

Examinations.—The lesions of poikilocytosis noted on the 12th day, and again 10 days later. On the 24th day two points were present, but no piroplasms were noticed.

The animal recovered.

5. *Mule* 2324.—Aged Argentine gelding.

Injected as above.

Temperature.—Very slight reaction from the 8th day and lasting for 10 days.

Examinations.—On the day of the maximum temperature of 102—12 days after injection—three points were present; three days later one piroplasm was noted for the first time. Another piroplasm was present the following day, and two noted on the 19th day. All further examinations negative, and were discontinued from the 27th day.

6. *Mule* 2325.—Aged Argentine gelding.

Injected as above.

Temperature.—Reaction of a very slight nature from the 8th to 19th days. On the 24th day the temperature commenced to rise and indicated a second reaction, reaching 103.2 four days later.

Examinations.—The lesions of a slight poikilocytosis observed during the height of the first reaction, and on the 15th and 16th days points were present in fair numbers. Piroplasma equi not noted during either reaction.

Recovered.

Results.—Of six Argentine mules injected with blood of a Transvaal horse foal, 2nd generation, immune against piroplasma equi, all showed a reaction, and accompanied in four cases with piroplasms, and recovered.

Third Generation.

Transvaal horse foal injected with blood of Transvaal horse foal 2054 *—immune against piroplasma equi.*

7. *Horse foal* 2314.—Obtained from the S.A.C.

Injected on the 16th October, 1906, with 5 c.c. defibrinated fresh blood of foal 2054.

Temperature.—Fluctuated between 100 and 103 for the first 10 days, and on the evening of the 11th day reached 105, dropped to 99.6 on the morning of the 14th day, but returned to 105.6 in the evening of the 16th day. The 20th day marked the commencement of a second reaction lasting for 11 days, although not of as strong a nature as the first.

Examinations.—Piroplasms and rosettes present on the 10th day for the first time; the former increased in numbers the following day, but were only noted in rare numbers each day until the 19th. Piroplasms and points were occasionally noted during the next 20 days, and on the 39th day after injection the lesions of poikilocytosis were observed. All further examinations negative.

Argentine horses injected with blood of Transvaal foal 2054—*immune against piroplasma equi.*

Argentine horses 2231, 2242 were injected on the 16th October, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of Transvaal horse foal 2054.

8. *Horse* 2231.—Four-year-old Argentine mare.

Injected as above.

Temperature.—Very slight reaction between 100 and 102.4, lasting for 19 days. On the 22nd day after injection a strong curve was noted, the temperature recording 106.4 in the evenings of the 27th and 28th days, after which it slowly fell and regained normal on the 35th day after injection.

Examinations.—On the 7th day points were noted, the eyes were yellow, and the animal was generally weak, but the following day an improvement was shown. Points were again present on the 9th, 10th, and 11th days, and on the 15th day the lesions of poikilocytosis appeared. Piroplasms noted for the first time on the 16th day, and were present in rare numbers until the 24th day. Coinciding with the time of the maximum temperature during the second reaction, the animal was noticed to be weak; piroplasms were noted on the 28th and 29th days, but in rare numbers. Piroplasms, points, and rosettes were occasionally present during the next 14 days.

9. *Horse* 2242.—Six-year-old Argentine gelding.

Injected as above.

Temperature.—On the 3rd day a rise to 104 was noted, but on the 7th day it recorded 100, from when a reaction commenced, reaching 105 on the 12th day, and keeping high for a considerable time. 105.2 was recorded in the evenings of the 20th, 21st, 22nd, and 23rd days. The temperature now remained normal until the 36th day, when a rise from 100.4 in the morning to 105 in the evening was noted (on account of relaxity of the anus, the temperature record was probably not accurate). The animal died the following morning from broncho-pneumonia, probably contracted on board ship.

Examinations.—One piroplasm seen for the first time on the 7th day, when the eyes were noted to be slightly yellow, and the animal was generally weak. This weakness was not so pronounced the following day, and on the 10th day from injection piroplasms were again noted accompanied with points. Rings, piroplasms, and points were noted almost daily for the next 20 days. The animal remained in poor condition during the whole of the reaction, with petechial spots in the eyes and pallid mucous membranes. The lungs were also affected.

Post-mortem.—

Condition:—Good.

Lungs:—Apex and lower edge of right lung and also lower edge of left lung shows broncho-pneumonia (red and grey hepatisation). A few patches of yellow fibrinous lymph on surface of right lung.

Heart:—Normal.

Liver:—Congested.

Kidneys:—Congested.

All other organs normal.

Result.—Of one Transvaal horse foal and two Argentine horses injected with blood of a Transvaal foal, immune against piroplasma equi, 2nd generation, all showed a reaction accompanied with piroplasma equi, and one Argentine horse died from broncho-pneumonia—probably ship's pneumonia and contracted on board ship.

Transvaal mules injected with blood of Transvaal horse foal 2054—*immune against piroplasma equi.*

10. *Mule* 2216.—Three-year-old gelding.

Injected on the 16th October, 1906, with 5 c.c. defibrinated fresh blood of foal 2054.

Temperature.—Very slight reaction, only recording a maximum of 101.4 on the 14th day.

Examinations.—Points present on the 10th day, and the following morning piroplasms, in rare numbers, noted for the first time. Piroplasms were also present in rare numbers on the 15th, 16th, 17th, and 18th days. The lesions of poikilocytosis noticed on the 21st and 22nd days, but all further examinations proved negative.

Transvaal mules 2218, 2219, 2221 and 2232 were injected subcutaneously on 21st September, 1906, with 5 c.c. fresh defibrinated blood of Transvaal horse foal 2054.

11. *Mule* 2218.—Three-year-old Transvaal gelding.

Injected as above.

Temperature.—A slight reaction from the 18th day, continuing until discharge.

Examinations.—A few rings noted on the 14th day, remaining for the following two days. The lesions of a slight poikilocytosis noticed on the 18th day and again on the 23rd day. Poikilocytosis and points were occasionally noted until the 32nd day, but no piroplasms were seen.

12. *Mule* 2219.—Three-year-old Transvaal mare.

Injected as above.

Temperature.—Reaction commenced on the 7th day, reaching 103 in the evening of the same day. A second reaction followed from the 15th day lasting for 16 days, and reaching the maximum temperature of 103 on the 21st day after injection.

Examinations.—Rings noted on the 14th, 15th, and 16th days and a few points on the 26th day. The lesions of poikilocytosis observed on the 35th and 27th days, but no piroplasms.

13. *Mule* 2221.—Three-year-old Transvaal gelding.

Injected as above.

Temperature.—Reaction from the 9th day, but of a very indistinct nature, and lasting until the 26th day after the injection.

Examinations.—One piroplasm noted for the first time on the 12th day; they increased and became fairly frequent on the 14th and 15th days; on the former date—the 14th—rods were also present. Piroplasms were again noted on the 18th and 22nd days, and on the 25th day the lesions of poikilocytosis were

observed. Poikilocytosis also present on the 26th day, accompanied with points, and the latter were again noted on the following day.

14. *Mule* 2232.—Three-year-old Transvaal mare.

Injected as above.

Temperature.—A sharp rise noted from the date of injection, reaching 104 in the evening of the 4th day, but recovered to normal, and from the 10th day a reaction of a rather indistinct nature ensued, reaching 103 on the 14th day, and remaining between this figure and 100 for 18 days.

Examinations.—Rings noted on the 14th day in fair numbers, and were also present on the 15th day, when they were accompanied with rods. One piroplasm noted for the first time on the 18th day, together with the lesions of a slight poikilocytosis. Poikilocytosis was also noted on the 22nd day, and two days later another piroplasm was present. Points were noticed the following day, and on the 26th day piroplasms and points were present in fair numbers.

Inoculation of Argentine mules with blood of Transvaal foal 2054—*immune against piroplasma equi.*

Argentine mules 2368, 2369, 2370, 2371 and 2372 were all injected subcutaneously on the 26th November, 1906, with 5 c.c. defibrinated fresh blood of Transvaal horse foal 2054.

15. *Mule* 2368.—Aged Argentine gelding.

Injected as above.

Temperature.—Reaction of a distinct character, lasting from the 7th to the 18th day, and reaching the maximum temperature of 102.4 on the evening of the 7th day.

Examinations.—All negative.

16. *Mule* 2369.—Eighteen-month-old stallion imported from the Argentine.

Injected as above.

Temperature.—Reaction of an indistinct nature, reaching 102.4 on the 7th day, and returning to normal on the 15th day.

Examinations.—Piroplasms present for the first time in rare numbers on the 14th day, accompanied with the lesions of poikilocytosis. Slight poikilocytosis observed three days later. All further examinations negative.

17. *Mule* 2370.—Six-year-old Argentine gelding.

Injected as above.

Temperature.—Reaction from the 9th to the 19th day, recording the maximum of 102.8 on the 14th day.

Examinations.—The lesions of poikilocytosis noticed on the 20th day. All other examinations negative.

18. *Mule* 2371.—Four-year-old Argentine gelding.

Injected as above.

Temperature.—No distinct reaction.

Examination.—Slight poikilocytosis noted on the 14th day, and three days later piroplasms seen for the first time All further examinations negative.

19. *Mule* 2372.—Eighteen-month-old Argentine mare.

Injected as above.

Temperature.—Sharp reaction from the 4th day, lasting for four days, and recording between 103 and 104. A slight reaction noted from the 10th to the 18th days, but only reaching 102.8.

Examinations.—Piroplasms seen for the first time on the 18th day, and in rare numbers three days later. Rosette forms noted on the 20th day.

Argentine mules 2514, 2518, 2519, 2520, 2522, 2523, 2524, 2525, 2531, 2532, 2548 were all injected subcutaneously on the 30th January, 1907, with 5 c.c. fresh defibrinated blood of foal 2054.

20. *Mule* 2514.—Argentine gelding.

Injected as above.

Temperature.—Reaction from the 11th day, lasting for 10 days, and recording the maximum temperatures of 104 and 104.4 on the 17th and 18th days respectively.

Examinations.—All negative.

21. *Mule* 2518.—Argentine mare.

Injected as above.

Temperature.—Reaction from the 5th day, reaching a maximum of 104.2 in the evening of the 11th day, and remaining about this record for the next seven days.

Examinations.—All negative.

22. *Mule* 2519.—Argentine mare.

Injected as above.

Temperature.—Sharp rise from the 4th day, reaching 103.6 24 hours later. Reaction of an indistinct nature from the 10th to the 21st days, the evening record on this latter date being 103.6.

Examinations.—All negative.

23. *Mule* 2520.—Argentine gelding.

Injected as above.

Temperature.—At the date of injection the temperature recorded 105, but slowly fell to 101 on the 4th day, rising again to 104 on the 6th day. On the morning of the 11th day the temperature was 100.6, rising in the evening to 104. It now fell and remained normal.

Examinations.—All examinations negative.

24. *Mule* 2522.—Argentine mare.

Injected as above.

Temperature.—Very slight reaction, the temperature recording the maximum of 103 on the 13th day.

Examinations negative.

25. *Mule* 2523.—Argentine stallion.

Injected as above.

Temperature.—Slight reaction from the 7th day, reaching 102.8 six days later.

Examinations.—Slight poikilocytosis noted on the 13th day. All other examinations negative.

26. *Mule* 2524.—Argentine gelding.

Injected as above.

Temperature.—Very slight reaction.

Examinations.—All negative.

27. *Mule* 2525.—Argentine mare.
Injected as above.
Temperature.—Very slight reaction.
Examinations.—All negative.

28. *Mule* 2531.—Argentine gelding.
Injected as above.
Temperature.—Reaction from the 7th day, reaching 103 on the 11th and 12th days.
Examinations.—All negative.

29. *Mule* 2532.—Argentine gelding.
Injected as above.
Temperature.—Reaction from the 8th day, lasting 15 days, and reaching a maximum of 104.4 fourteen days after injection.
Examinations.—The lesions of poikilocytosis noted on the 15th day. All other examinations negative.

30. *Mule* 2548.—Argentine mare.
Injected as above.
Temperature.—Sharp rise to 104 on the 1st day after injection, and from the 8th day a slight reaction was noticed lasting for 12 days. A second slight reaction to 104.2 was noted on the 23rd day, but only lasted for six days.
Examinations.—All negative.

Argentine donkeys injected with blood of Transvaal foal 2054—*immune against piroplasma equi.*

Argentine donkeys 2248, 2249, 2250 and 2251 were injected on the 26th November, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of Transvaal horse foal 2054.

31. *Donkey* 2248.—Five-year-old Argentine mare.
Injected as above.
Temperature.—Slight reaction, the temperature remaining between 98 and 102 for 21 days after injection.
Examinations.—All negative.

32. *Donkey* 2249.—Four-year-old Argentine gelding.
Injected as above.
Temperature.—Very slight reaction.
Examinations.—One point noted on the 15th day. All other examinations negative.

33. *Donkey* 2250.—Three-year-old Argentine mare.
Injected as above.
Temperature.—Slight reaction.
Examinations.—One piroplasm seen on the 18th day. All other examinations negative.

34. *Donkey* 2251.—Four-year-old Argentine gelding.
Injected as above.
Temperature.—Slight reaction.
Examinations.—Piroplasms seen on the 15th day only, but in very rare numbers.

Argentine donkeys 2256, 2257 and 2258 were all injected subcutaneously on the 16th October, 1906, with 5 c.c. defibrinated fresh blood of Transvaal horse foal 2054,

35. *Donkey* 2256.—Five-year-old Argentine mare.

Injected as above.

Temperature.—On the 4th day after injection the temperature rose from 99.8 in the morning to 103.4 in the evening. It remained high for the next three days, and the donkey died of debility. On *post-mortem*, anaemia, fatty degeneration and hydronephrosis was found.

Note.—Slipped her foal during the reaction.

36. *Donkey* 2257.—Three-year-old Argentine mare.

Injected as above.

Temperature.—Slight reaction, from the 7th day, lasting for 11 days, and recording as a maximum 102.8 in the evening of the 10th day after injection.

Examinations.—Piroplasms in rare numbers noted for the first time on the 10th day, points having been noticed two days previously. Piroplasms and the lesions of poikilocytosis were noted almost daily from the 10th to 24th days.

37. *Donkey* 2258.—Two-year-old Argentine mare.

Injected as above.

Temperature.—A slight reaction from the 9th to the 19th day. A second slight reaction from the 26th to the 35th days, touching 103 on the 30th day after injection.

Examinations.—One piroplasm seen for the first time on the 10th day, and piroplasms again noted two days later, accompanied with points. The lesions of poikilocytosis appeared on the 14th day, and piroplasms were noted daily from the 15th to 18th days. Piroplasms present in very rare numbers on the 21st and 23rd days, and on the 24th day from injection the lesions of poikilocytosis were again noticed.

Results of 5 Transvaal mules, 16 Argentine mules and 7 Argentine donkeys injected with blood of a Transvaal horse foal—immune against piroplasma equi (2nd generation).

All passed through a piroplasmosis reaction, and, with the exception of 1 donkey which died of debility, all recovered.

Experiment No. 6.

Fourth Generation.

Transvaal Horse Foal 2314.—Transvaal horse foal 2314 had been injected with blood of foal 2054 on the 16th October, 1906 [compare Experiment No. 5 (7)].

Injection of Transvaal horse foal with blood of Transvaal horse foal 2314—immune against piroplasma equi.

1. *Horse Foal* 2273.—Four-month-old foal, and born on the station.

Injected on the 10th December, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of foal 2314.

Temperature.—Reaction from the 3rd day, reaching 104.2 on the 6th day, and regaining normal 16 days after injection. A second reaction noted from the 22nd day, reaching 105 in the evening five days later, when the temperature fell and remained from the 35th day after injection.

Examinations.—Piroplasms noted for the first time on the 11th day, and were present on the following two days. Again noted on the 15th day, accompanied with a slight poikilocytosis, the latter being also noticed on the 19th day. Points were present the following day, and, together with piroplasms, were frequently noted during the next 10 days. One rosette also seen on the 45th day.

Argentine mules injected with blood of Transvaal foal 2314, *immune against piroplasma equi.*

2. *Mule* 2448.—Argentine gelding. (Had been in a horse-sickness experiment previously.)

Injected subcutaneously on the 14th February, 1907, with 3 c.c. fresh defibrinated blood of foal 2314.

Temperature.—Slight reaction from the 6th day, reaching 102.8 on the 10th day, and remaining normal from the 14th day after injection.

Examinations.—All negative.

Note.—The following Argentine mules—Nos. 2484, 2486, 2487, 2488, 2498, 2490, 2491, 2492, 2493, 2500, 2501, 2502—had been running on a farm infected with piroplasmosis, and, in addition, had been utilised for horse-sickness experiments before the injection of 3 c.c. defibrinated fresh blood of Transvaal horse foal 2314 on the 7th February, 1907.

3. *Mule* 2484.—Four-year-old Argentine gelding.

Injected as above.

Temperature.—Slight reaction.

4. *Mule* 2486.—Three-and-a-half-year-old mare, imported from the Argentine.

Injected as above.

Temperature.—Reaction from the 4th day, recording 105 three days later, and for the next four days the evening temperature remaining about 104. A second reaction noted from the 21st day, lasting for 10 days, and reaching the maximum temperature of 104 on the 25th day after injection.

5. *Mule* 2487.—Three-and-a-half-year-old Argentine gelding.

Injected as above.

Temperature.—Reaction from the 10th day, reaching 103.8 three days later. A sharp rise was noted from the 19th day, rising to 103.2 the following day, falling to 100 in the morning of the 22nd day, and rising in the evening to 104.2.

6. *Mule* 2488.—Argentine mare, about three and a half years old.

Injected as above.

Temperature.—No distinct reaction until the 24th day after injection, when the temperature rose from 99.6 in the morning to 103.6 in the evening, and remaining between 103 and 104 for the next seven days.

Examinations.—All negative.

7. *Mule* 2489.—Two-and-a-half-year-old Argentine mare.

Injected as above.

Temperature.—No distinct reaction until the 20th day after injection, when the temperature rose from 100 and recorded 105.4 in the evening of the 22nd day. It now fell, and 100.2 was noted in the morning of the 26th day, and 106 in the evening of the 29th day.

Examinations.—All negative.

8. *Mule* 2490.—Seven-year-old Argentine gelding.
   Injected as above.
   Temperature.—Slight reaction.

9. *Mule* 2491.—Five-year-old Argentine gelding.
   Injected as above.
   Temperature.—Very slight reaction, recording 103.4 on the 10th day from injection.

10. *Mule* 2492.—Aged Argentine gelding.
    Injected as above.
    Temperature.—Reaction from the 9th day, recording 104 on the 17th and 18th days. A second reaction from the 25th day, and reaching the same figure on the 27th and 28th days.

11. *Mule* 2493.—A three-and-a-half-year-old Argentine mare.
    Injected on the 7th February, 1907, subcutaneously with 3 c.c. defibrinated fresh blood of foal 2314.
    Temperature.—Slight reaction.

12. *Mule* 2500.—Argentine gelding.
    Injected as above.
    Temperature.—Slight reaction, reaching 103 on the 18th and 19th days.

13. *Mule* 2501.—Argentine gelding.
    Injected as above.
    Temperature.—Reaction, reaching 104.8 on the 21st and 22nd days.

14. *Mule* 2502.—Argentine mare.
    Injected as above.
    Temperature.—Slight reaction, reaching the maximum of 104.4 on the 24th day.

Mules 2503, 2504, 2505, 2506, 2507 injected subcutaneously with 3 c.c. defibrinated fresh blood of Transvaal horse foal 2314 on the 14th February, 1907.

15. *Mule* 2503.—Argentine gelding.
    Injected as above.
    Temperature.—Reaction, recording 104.8 on the 20th day.

16. *Mule* 2504.—Argentine mare.
    Injected as above.
    Temperature.—Reaction, reaching 104 on the 4th day after injection. A second reaction noted from the 27th to 32nd days.

17. *Mule* 2506.—Argentine mare.
    Injected as above.
    Temperature.—Reaction from the 14th day, and recording the maximum of 104.2 on the 24th day after injection.

18. *Mule* 2507.—Argentine gelding.
    Injected as above.
    Temperature.—Reaction from the 7th day, reaching 103.4 on the 11th day. Second reaction from the 14th day, lasting for 11 days.

Note.—The following mules, all directly imported from the Argentine, were injected on the 12th February, 1907, subcutaneously with 3 c.c. defibrinated fresh blood of foal 2314:—Mules Nos. 2581, 2588, 2537, 2589, 2590, 2591, 2592, 2594, 2595, and 2596. Further, they had been previously utilised for horse-sickness experiments.

19. *Mule* 2581.—Three-and-a-half-year-old Argentine mare.
Injected as above.
Temperature.—Reaction from the 7th day.

20. *Mule* 2588.—Two-and-a-half-year-old Argentine mare.
Injected as above.
Temperature.—No distinct reaction.

21. *Mule* 2537.—Argentine stallion.
Injected as above.
Temperature.—Reaction from the 9th day.

22. *Mule* 2589.—Two-and-a-half-year old mare.
Injected as above.
Temperature.—Reaction from the 13th to 23rd days, and reaching the maximum of 105.2 on the 20th day.
Examinations.—All negative.

23. *Mule* 2590.—Four-and-a-half-year-old Argentine mare.
Injected as above.
Temperature.—No reaction.

24. *Mule* 2591.—Two-and-a-half-year-old Argentine mare.
Injected as above.
Temperature.—Slight reaction from the 15th day, and recording the maximum of 103.8 in the 26th day after injection.

25. *Mule* 2592.—Three-and-a-half-year-old Argentine mare.
Injected as above.
Temperature.—Reaction from the 10th day, and recording 104.6 on the 22nd day. A secondary reaction from the 30th day.

26. *Mule* 2594.—Argentine gelding.
Injected as above.
Temperature.—Reaction from the 5th day; second from the 10th day.

27. *Mule* 2595.—Two-and-a-half-year-old Argentine mare.
Injected as above.
Temperature.—Doubtful reaction.

28. *Mule* 2596.—Two-and-a-half-year-old Argentine mare.
Injected as above.
Temperature.—Slight reaction.

The following Argentine mules were all injected on the 29th January, 1907, subcutaneously with 5 c.c. defibrinated fresh blood of foal 2314:—Nos. 2526, 2527, 2528, 2529, 2573, 2574, 2575, 2576, 2577.

29. *Mule* 2526.—Argentine stallion.
Injected as above.
Temperature.—Reaction from the 6th day.

30. *Mule* 2527.—Argentine mare.
Injected as above.
Temperature.—Slight reaction from the 8th day.

31. *Mule* 2528.—Argentine gelding.
Injected as above.
Temperature.—Reaction from the 8th day.
Examinations.—All negative.

32. *Mule* 2529.—Argentine gelding.
Injected as above.
Temperature.—Reaction from the 10th day.
Examinations.—All negative.

33. *Mule* 2573.—Argentine mare.
Injected as above.
Temperature.—Doubtful reaction.
Examinations.—Piroplasma equi noted in rare numbers on the 14th and 15th days.

34. *Mule* 2574.—Argentine mare.
Injected as above.
Temperature.—Reaction from the 8th day.
Examinations.—Piroplasma equi noted for the first time, but in rare numbers on the 19th day after injection.

35. *Mule* 2575.—Argentine mare.
Injected as above.
Temperature.—Reaction from the 7th day.
Examinations.—Slight poikilocytosis noticed on the 14th and 15th days.

36. *Mule* 2576.—Argentine mare.
Injected as above.
Temperature.—Slight reaction from the 6th day.
Examinations.—Piroplasms noted on the 13th day only.

37. *Mule* 2577.—Argentine mare.
Injected as above.
Temperature.—Distinct reaction from the 7th day, recording 103.6 on the 14th and 15th days.
Examinations.—Piroplasms in rare numbers noted for the first time on the 13th day, and the following two days were again present, accompanied with the lesions of poikilocytosis.

Result of 1 Transvaal horse foal and 36 Argentine mules injected with blood of a Transvaal horse foal, immune against piroplasma equi (third generation).

All passed through a reaction and recovered.

Experiment No. 7.

Fifth Generation.

Transvaal horse foal injected with blood of a Transvaal horse foal, immune against piroplasma equi.

1. *Horse Foal* 2274.—Filly born on the station in September, 1906.
Injected on the 28th January, 1907, subcutaneously with 5 c.c. defibrinated fresh blood of foal 2273,

Temperature.—Reaction from the 6th day, reaching 103.6 on the 10th day. A second reaction from the 17th day, lasting for 10 days.

Examinations.—All negative.

Note.—The following Argentine mules were all injected on the 29th January, 1907, subcutaneously with 5 c.c. defibrinated fresh blood of foal 2273, now immune against piroplasma equi:—Nos. 2530, 2533, 2566, 2567, 2568, 2569, 2570, 2571 and 1572.

2. *Mule* 2530.—Argentine mare.

Injected as above.

Temperature.—Reaction from the 6th day.

3. *Mule* 2533.—Argentine mare.

Injected as above.

Temperature.—The morning record on the 7th day was 97.8, rising to 101.4 in the evening of the 9th day, but dropped during the night, and the animal died the following morning from pneumonia.

4. *Mule* 2566.—Argentine mare.

Injected as above.

Temperature.—Reaction from the 7th day, lasting for 14 days.

Examinations.—Slight poikilocytosis noted on the 12th and 13th days. Piroplasma equi present in rare numbers on the 14th day.

5. *Mule* 2567.—Argentine mare.

Injected as above.

Temperature.—Reaction from the 6th day, recording 105.6 in the evening of the 14th day.

Examinations.—Slight poikilocytosis noted on the 11th day, and the following day piroplasma equi present. Piroplasms again present in rare numbers on the 15th day.

6. *Mule* 2568.—Argentine gelding.

Injected as above.

Temperature.—Reaction from the 7th day.

Examinations.—Piroplasma equi noted on the 14th day, and the lesions of poikilocytosis present the following day.

7. *Mule* 2569.—Argentine mare.

Injected as above.

Temperature.—Reaction from the 5th day, recording 103.6 four days later.

Examinations.—All negative.

8. *Mule* 2570.—Argentine mare.

Injected as above.

Temperature.—Reaction from the 4th day. Second rise noted from the 14th to 22nd days.

Examinations.—Piroplasma equi noted on the 12th day, and the lesions of poikilocytosis the following day. Piroplasma equi again noted during the second reaction, and followed on the 19th day by the lesions of a slight poikilocytosis.

9. *Mule* 2571.—Argentine gelding.

Injected as above.

Temperature.—Reaction from the 4th day, lasting for 25 days, and recording between 100 and 105.2, this latter record being noted on the 19th day.

Examinations.—Piroplasma equi noted on the 13th, 14th and 15th days; on this latter date the lesions of poikilocytosis also being present. On the 14th day the urine was coloured red, but became clear two days later. On the 15th day the red corpuscles numbered 3,700,000 per c.m.m., and 24 hours later decreased to 2,500,000. Slight poikilocytosis noted on the 18th and 19th days, and again on the 24th day.

10. *Mule* 2572.—Argentine mare.

Injected as above.

Temperature.—Reaction from the 5th day, reaching 104.6 in the evening four days later, and regaining normal on the 14th day. A second reaction from the 12th day, and lasting for six days.

Examinations.—Piroplasms present in fair numbers on the 13th day, and again noted on the following two days.

Result of 1 Transvaal horse foal and 9 Argentine mules injected with blood of a Transvaal horse foal, immune against piroplasma equi.

All passed through a reaction, and, with the exception of one Argentine mule, which died of pneumonia, recovered.

Note.—In the majority of cases these reactions were of a severe character; secondary reactions were also noted, and piroplasma equi was frequently present.

Experiment No. 8.

Sixth Generation.

Injection with blood of Transvaal foal 2274, immune against piroplasma equi. (Compare Experiment 7, 1.)

Transvaal Horse Foal 2621.—Colt obtained from the S.A.C.

Injected on the 26th March, 1907, subcutaneously with 5 c.c. defibrinated blood of foal 2274.

Temperature.—Reaction from the 6th day.

Examinations.—The lesions of poikilocytosis noted on the 11th, 14th and 15th days, and on the 16th day piroplasma equi appeared.

Result.—One Transvaal horse foal inoculated with blood of a horse foal, immune against piroplasma equi (fifth generation), passed through a piroplasmosis reaction and recovered.

Experiment No. 8a.

Seventh Generation.

Transvaal horse foal injected with blood of Transvaal foal 2621, *immune against piroplasma equi.*

Transvaal Horse Foal 2734.—

Injected subcutaneously on the 25th April, 1907, with 10 c.c. blood of foal 2621.

Temperature.—Reaction from the 8th day. Second reaction from the 26th day, recording 105.6 on the 39th day.

Examinations.—Piroplasma equi noted on the 9th day, and again on the 40th day.

Argentine horse injected with blood of Transvaal foal 2621, *immune against piroplasma equi.*

Horse 2684.—Four-year-old Argentine mare.

Injected on the 8th May, 1907, with 5 c.c. defibrinated fresh blood of foal 2621.

Temperature.—Reaction from the 7th day, rising to 104.2 in the evening of the 10th day, falling to sub-normal 24 hours later, and recording 97. On the 13th day temperature reached 105.6 in the evening, but the animal died the following morning from debility, complicated with piroplasmosis.

Post-mortem Examination.—

Condition:—Poor; blood-stained foam in nostrils; flesh of a brick-red colour.

Lungs:—Slight œdema; liquid in peritoneal cavity.

Heart:—Inbibition of left and right endocards, myocard soft; abnormal amount of blood-stained liquid in pericard.

Spleen:—Slightly enlarged; pulpa soft.

Liver:—Decomposed.

Kidneys:—Pale and yellowish.

Stomach:—Mucosa pale.

Intestines:—Cæcum pale and slate coloured; colon slate coloured; strongylus armatus and tetracanthus present.

Result.—One Transvaal horse foal and one Argentine mare injected with blood of a Transvaal horse foal (sixth generation); both passed through a typical piroplasmosis reaction, the Argentine mare dying of debility, complicated with piroplasmosis.

Experiment No. 8b.

Eighth Generation.

Transvaal horse foal injected with blood of Transvaal foal 2734, *now immune against piroplasma equi.*

Horse Foal 2786.—Transvaal gelding.

Injected subcutaneously on the 30th January, 1907, with 5 c.c. blood of foal 2734.

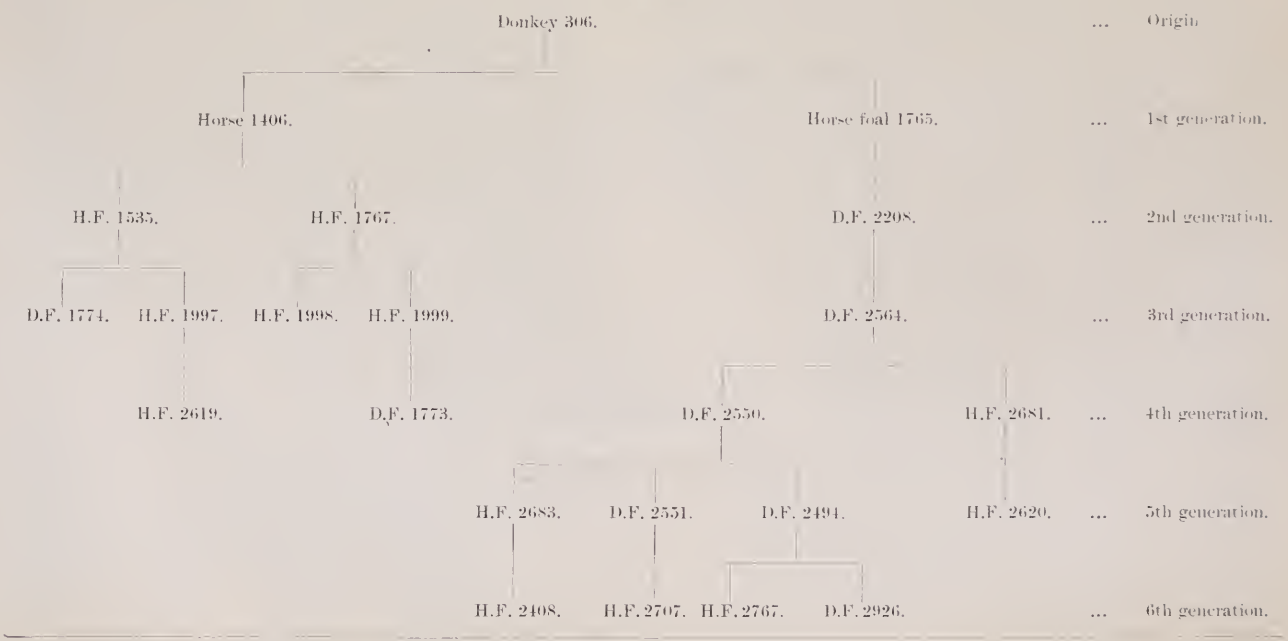
Temperature.—Reaction from the 4th day. Second reaction from the 23rd day, and lasting for 11 days.

Examinations.—Poikilocytosis noted on the 5th, 6th, 11th and 12th days, and piroplasma equi on the 7th, 8th and 9th days.

Result.—One Transvaal horse foal injected with blood of a Transvaal horse foal, immune against piroplasma equi (seventh generation), passed through a piroplasmosis reaction and recovered. A second reaction was also noticed.

3. Inoculation against Equine Piroplasmosis by means of Horse Foal and Donkey Foal Blood.

Origin Donkey Blood.



3.—Inoculation by Means of Donkey Foal and Horse Foal Blood. Origin Donkey Blood.

Donkey 306. (Compare Annual Report 1904-5, page 101.)

Experiment No. 9.

First Generation.

1. *Horse* 406.—About three years old, injected with 10 c.c. blood of donkey 306. Compare Annual Report 1905-6, page 90.

2. *Horse foal* 1765.—

Injected on the 21st April, 1906, subcutaneously with 5 c.c. blood of donkey 306.

Result.—Reaction from the 9th day, reaching 103 five days later and remaining normal from the 19th day. A slight rise noted from the 44th day, recording 103.4 the following day. Piroplasms noted for the first time, but in exceedingly rare numbers, on the 18th day. Again present on the 21st day, and together with rosettes, rings and points were occasionally noted during the next three weeks. At the time of the sharp rise on the 55th day, the mucous membranes were noted to be dirty and slightly injected.

Red Corpuscles.—

Count on 7th day	...	..	8,900,000	per c.m.m.
,, 10th ,,	...	...	9,190,000	,,
,, 12th ,,	...	...	7,776,000	,,
,, 14th ,,	...	...	9,040,000	,,
,, 20th ,,	...	...	8,496,000	,,
,, 22nd ,,	...	...	7,520,000	,,
,, 25th ,,	...	...	7,240,000	,,
,, 28th ,,	...	...	7,904,000	,,

Argentine horses injected with blood of donkey 306—*immune against piroplasma equi.*

Argentine horses 2229, 2231, 2238, 2239, 2243, 2244 and 2245.—These animals had previously been inoculated with blood of a foal immune against piroplasma equi, and were also utilised for horse-sickness experiments. They were now tested on their immunity against piroplasma equi.

Argentine horses 2229 and 2245 previously inoculated with blood of Transvaal foal 1535—compare Experiment 11, Nos. 7 and 8—and now injected on the 21st December, 1906, subcutaneously with 5 c.c. blood of donkey 306.

3. *Horse* 2229.—Three-year-old Argentine gelding.

Injected as above.

Result.—Reaction from the 7th day. All examinations negative.

4. *Horse* 2245.—Five-year-old Argentine gelding.

Injected as above.

Result.—Reaction from the 14th day, recording 105 in the following evening. A few points noted on the 5th day.

Argentine horses 2231, 2238, and 2239, previously inoculated with blood of Transvaal horse foals [compare Experiments 5 (8) and 10 (27

and 28)] now tested on their immunity. Injected on the 19th December, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of donkey 306.

5. *Horse* 2231.—Four-year-old Argentine mare.
   Injected as above.
   Result.—Slight reaction from the 13th day. All blood examinations negative.

6. *Horse* 2238.—Argentine three-year-old gelding.
   Injected as above.
   Result.—Slight reaction. One point present on the 16th day.

7. *Horse* 2239.—Seven-year-old gelding.
   Injected as above.
   Result.—Slight reaction. One point present on the 16th day.

Argentine horses 2243 and 2244 previously injected with blood of foal 2208 (compare Experiment 10, Nos. 30 and 29) and now injected on the 12th December, 1906, subcutaneously with 5 c.c. fresh defibrinated blood of donkey 306.

8. *Horse* 2243.—Four-year-old Argentine gelding.
   Injected as above.
   Result.—Slight reaction from the 12th day. All examinations negative.

9. *Horse* 2244.—Argentine gelding.
   Injected as above.
   Result.—Slight reaction. Poikilocytosis and a few points noted.

Argentine mules injected with blood of donkey 306—*immune against piroplasma equi.*

Argentine mules Nos. 2322 and 2325 were previously injected on the 5th November, 1906, with blood of foal 2053, immune against piroplasma equi (compare Experiment 5, Nos. 4 and 6), and mule 2326 was injected on the same date with blood of foal 1999, immune against piroplasma equi (compare Experiment 12, No. 46), now tested on their immunity; three were injected on the 19th December, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of donkey 306.

10. *Mule* 2322.—Two-and-a-half-year-old Argentine gelding.
    Injected as above.
    Result.—Slight reaction from the 15th day, piroplasms and the lesions of a slight poikilocytosis noted on the 11th and 18th days. Slight poikilocytosis again present on the 21st day.

11. *Mule* 2325.—Aged Argentine gelding.
    Injected as above.
    Result.—Slight reaction. Poikilocytosis and points noted on the 13th day; the former again present on the 23rd day Piroplasma equi not present.

Argentine donkeys, injected with blood of donkey 306—*immune against piroplasma equi.*

Donkeys 2248 and 2249 were injected on the 26th November, 1906, with blood of foal 2054, immune against piroplasma equi, and donkey 2254 was injected on the same date with blood of foal 1997,

immune against piroplasma equi. All three donkeys were now injected on the 19th December, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of donkey 306.

13. *Donkey* 2248.—Four-year-old Argentine mare.

Injected as above.

Result.—Reaction from the 11th day. Piroplasms present on the 20th and 22nd days.

14. *Donkey* 2249.—Four-year-old Argentine gelding.

Injected as above.

Result.—Slight reaction from the 15th day, reaching 103 twenty-four hours later. All microscopical examinations negative.

15. *Donkey* 2254.—Three-year-old Argentine mare.

Injected as above.

Result.—Reaction from the 15th day. Piroplasms present on the 16th day.

Results.—One Transvaal horse foal injected with blood of a Transvaal donkey, immune against piroplasma equi, passed through a reaction and recovered. Seven Argentine horses, three Argentine mules, and three Argentine donkeys were tested on their immunity, and all showed slight reactions and recovered.

Experiment No. 10.

Second Generation.

Transvaal horse foal injected with blood of Argentine horse—immune against piroplasma equi.

1. *Transvaal Foal* 1535.—About seven months old. (Note.—This foal was utilised in a horse-sickness experiment in January, 1906.)

Injected subcutaneously on the 19th April, 1906, with 5 c.c. blood of Argentine horse 1406.

Temperature.—Reaction from the 8th day, rising to 105.6 sixteen days after injection, and remaining high for the next four days. The temperature remained normal from the 28th day until the 37th day, when a rise from 100 in the morning to 104.8 in the evening was noted.

Examinations.—Piroplasms noted for the first time, but in rare numbers, on the 18th day, and again on the 22nd day. Piroplasms, rosettes, marginal points, and the leaf form were occasionally noted until the 54th day after injection. The mucous membranes were yellow on the 28th day, and slightly pale on the 45th day. On the 37th day, the occasion of the sharp rise referred to above, the animal was affected with a nasal catarrh.

Red Corpuscles.—The blood count on the 9th day recorded 8,943,000, falling to 7,232,000 four days later, and on the 21st day recorded 5,568,000 per c.m.m.

2. *Foal* 1767. (Note.—This animal had been utilised in a horse-sickness experiment in January, 1906.)

Injected on the 19th June, 1906, subcutaneously with 5 c.c. fresh defibrinated blood of horse foal 1406.

Temperature.—Reaction from the 7th day, reaching 104 in the evening four days later, but falling to 100 on the 18th day. A short rise noted from the 23rd to 27th days, but only reaching 103.

Examinations.—The pear and ring form of piroplasma equi noted on the 13th day, and a few more seen seven days later. Piroplasms were frequently noted during the next ten days, but they appeared for the last time on the 34th day. Points were noted on the 34th day, and again on the 50th day.

Red Corpuscles.—

Count of 9th day	...	...	9,600,000 per c.m.m.
,, 13th ,,	...	...	6,320,000 ,,
,, 15th ,,	...	...	5,600,000 ,,
,, 19th ,,	...	...	6,816,000 ,,
,, 21st ,,	...	...	6,260,000 ,,
,, 23rd ,,	...	...	6,400,000 ,,
,, 26th ,,	...	...	5,610,000 ,,
,, 29th ,,	...	...	7,260,000 ,,

Transvaal donkey foal injected with blood of horse foal 1765—*immune against piroplasma equi.*

3. *Donkey Foal* 2208.—Six months old and born on the station.

Injected on the 14th September, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of horse foal 1765.

Temperature.—Reaction from the 10th day, but only recording as a maximum 103.2 on the 17th day. Second reaction noted on the 32nd day, recording 103 six days later.

Examinations.—All negative.

Argentine horses injected with blood of Transvaal horse foal 1765—*immune against piroplasma equi.*

4. *Horse* 2073.—Two-year-old Argentine mare.

Injected subcutaneously on the 9th July with 5 c.c. defibrinated fresh blood of foal 1765.

Temperature.—Slight reaction from the 11th to 20th days, the temperature recording 104.6 on this latter date. A second reaction from the 24th day, recording 105 six days later, and regaining normal on the 34th day.

Examinations.—Piroplasms noted, but in very rare numbers on the 12th day, accompanied with rings. Piroplasms present the following day, and again on the 17th day. Points noted on the 16th and 18th days; the mucous membranes pale on the 18th day. Piroplasms again noted during the second reaction, on the 27th day, and one point noted on the 31st day.

5. *Horse* 2074.—Two-year-old Argentine mare.

Injected subcutaneously on the 9th July, 1906, with 5 c.c. blood of foal 1765.

Temperature.—Very slight reaction. The animal died on the 31st day from rupture of spleen.

Examination.—Piroplasms noted for the first time on the 12th day, accompanied with rings and points; the latter were also present on the 16th and 17th days, and the following day piroplasms were again noted. One piroplasm and one point were present on the 21st day, and on the following day, when the animal died, smears were made of the spleen, but not piroplasms were seen.

Post-mortem.—

Condition:—Good; body tympanitic.

Lungs:—Several gallons of blood in peritoneal cavity.

Spleen:—Anterior surface shows a ragged rupture about three inches long; spleen enlarged about three times normal; pulpa dark brown, but firm.

All other organs normal in appearance, but pale.

Transvaal horse injected with blood of Transvaal horse foal 1765—*immune against piroplasma equi.*

6. *Horse* 2205.—Aged gelding. (Note.—This animal had previously been utilised in horse-sickness experiments.)

Injected on the 14th September subcutaneously with 5 c.c. defibrinated fresh blood of foal 1765.

Result.—No distinct reaction.

Results of animals inoculated with blood of a Transvaal horse foal—immune against piroplasma equi (first generation).

1 Transvaal donkey foal showed a reaction and recovered.

Of 2 Argentine horses one showed a reaction and recovered, and one died from rupture of spleen.

1 Transvaal horse—no distinct reaction.

2 Transvaal horse foals injected with blood of a Transvaal horse (first generation) showed reactions and recovered.

Experiment No. 11.

Third Generation.

Transvaal donkey foal injected with blood of Transvaal horse foal 1535—*immune against piroplasma equi.*

1. *Donkey Foal* 1774.—Eighteen-month-old mare, and born on station. (Note.—This donkey foal had been utilised in horse-sickness experiments in August, 1906.)

Injected on the 14th September, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of Transvaal horse foal 1535.

Result.—Reaction from the 12th day.

All examinations negative.

Transvaal horse foals injected with blood of Transvaal horse foal—immune against piroplasma equi.

Transvaal horse foals 1997, 1998 and 1999—all six-month-old mares—were injected on the 9th July, 1907, subcutaneously with 5 c.c. defibrinated fresh blood of Transvaal horse foal 1535.

2. *Horse Foal* 1997.—

Injected as above.

Result.—Reaction from the 26th to the 41st day. Piroplasms noted on the 15th, 16th, 19th, 20th and 27th days, and circular points noted on the 22nd day.

3. *Horse Foal* 1998.—

Injected as above.

Result.—Reaction from the 19th day. Piroplasms noted on the 13th, 15th, 16th, 21st, 22nd, 28th, 36th and 37th days.

4. *Horse Foal* 1999.—

Injected as above.

Result.—High temperature at date of injection. Reaction from the 13th day. Piroplasms present on the 15th, 18th and 21st days, and on this latter date were accompanied with rings and points.

Argentine horses injected with blood of Transvaal horse foal—immune against piroplasma equi.

Horses 2061 and 2062, both two-year-old Argentine mares, were injected on the 16th June, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of Transvaal horse foal 1535.

5. *Horse* 2061.—

Injected as above.

Result.—Reaction from the 14th day, reaching 104 on the 17th day and 105 on the 30th day. Piroplasms, rings and the lesions of poikilocytosis noted on the 18th day.

6. *Horse* 2072.—

Injected as above.

Result.—Reaction from the 11th day, recording 105 on the 11th, 13th and 14th days. The temperature now remained fairly high until the 22nd day, and two days later a sharp drop to subnormal of 97.6 was recorded. The animal now rallied, but after the temperature reached 105 on the 29th day, the animal was killed on account of pleuro-pneumonia. Piroplasms, accompanied with rings, noted on the 12th, 13th, 15th, 20th and 22nd days; mucous membranes pale; the animal very weak on the 18th day.

Note.—Horses 2229 and 2245 were inoculated with horse-sickness blood on the 3rd September, 1906, and now injected subcutaneously with 5 c.c. defibrinated fresh blood of foal 1535 on the 16th October, 1906.

7. Horse 2229.—Three-year-old gelding.

Injected as above.

Result.—Reaction from the 16th day, recording 105 on the 23rd, 24th and 25th days, and regaining normal on the 33rd day. Slight yellowish appearance of the eyes and general weakness noted on the 6th day, but followed by an improvement within 24 hours. Ring forms present on the 11th day, and piroplasms noted on the 12th, 14th and 15th (accompanied with the lesions of poikilocytosis on these latter two days), 16th, 17th, 18th, 19th (on which date one rosette was also noted), 22nd, 23rd and 24th. Points present on the 28th day, and eight days later piroplasms again observed.

The lowest records of the red corpuscle were noted on the 30th day—4,700,000, and on the 39th day 4,600,000 per c.m.m.

8. *Horse* 2245.—Three-year-old Argentine gelding.

Injected as above.

Result.—Reaction from the 10th day, and lasting for 20 days. Short reaction noted from the 32nd to 37th days. Points noted on the 7th day, accompanied with the lesions of weakness and yellowish eyes. Piroplasms noted on the 15th, 16th, 17th and 18th days, but on the latter two days in very rare numbers. The lesions of poikilocytosis, points and piroplasms occasionally noted from the 21st to 38th days. The red corpuscles reached the minimum record of 4,200,000 per cmm. on the 38th day.

Argentine donkeys injected with blood of Transvaal horse foal—immune against piroplasma equi.

Argentine donkeys 1846, 1847, 1848, 1849 and 1850, injected on the 16th June, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of foal 1535.

9. *Donkey* 1846.—Three-year-old Argentine mare.
   Injected as above.
   Result.—Slight irregular reaction.
10. *Donkey* 1847.—Three-year-old Argentine mare.
   Injected as above.
   Result.—Slight reaction.
11. *Donkey* 1848.—Four-year-old Argentine mare.
   Injected as above.
   Result.—Slight reaction.
12. *Donkey* 1849.—Two-year-old Argentine gelding
   Injected as above.
   Result.—Reaction from the 8th day.
13. *Donkey* 1850.—Three-year-old Argentine mare.
   Injected as above.
   Result.—(No record kept).

Argentine donkeys 2259, 2260, 2261, all three-year-old Argentine mares, and previously utilised for horse-sickness experiments, were injected on the 16th October, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of Transvaal horse foal 1535.

14. *Argentine Donkey* 2259.—
   Injected as above.
   Result.—Reaction from the 11th day. Chromatic points noted on the 5th and 7th days, and piroplasms—but in rare numbers—on the 15th, 16th and 17th days. The lesions of poikilocytosis present on the 16th, 21st and 26th days.
15. *Argentine Donkey* 2260.—
   Injected as above.
   Results.—Reaction from the 3rd day. Points noted on the 5th, 7th, 10th and 11th days; on this latter date piroplasms were also present. Piroplasms and the lesions of poikilocytosis occasionally noted until the 26th day.
16. *Argentine Donkey* 2661.—
   Injected as above.
   Result.—Irregular reaction. Piroplasms noted on the 10th, 11th, 14th, 15th, 17th and 21st days. Points present on the 16th day and the lesions of poikilocytosis appeared on the 22nd and 24th days.

Transvaal mules injected with blood of Transvaal horse foal—immune against piroplasma equi.

17. *Transvaal Mule* 2211.—Three-year-old mare, and previously utilised for horse-sickness experiments.
   Injected on the 16th October, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of foal 1535.
   Result.—Irregular reaction. Piroplasms and flagellated forms noted on the 10th day; the former again present on the 11th, 14th, 15th, 18th, 22nd and 23rd days.

Transvaal mules 2213, 2214, 2215 and 2217—previously utilised for horse-sickness experiments—were injected on the 21st September, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of Transvaal horse foal 1535.

18. *Mule* 2213.—Three-year-old Transvaal mare.

Injected as above.

Result.—Reaction from the 15th day, recording 106 nine days later. A second reaction commenced on the 32nd day, and terminated by the death of the animal seven days later from piroplasmosis (29th October, 1906). Piroplasms noted on the 13th, 15th and 16th days. Rings, points and the lesions of poikilocytosis occasionally noted. The pear-shaped piroplasm was observed on the 23rd day.

Post-mortem.—

Condition:—Fair; blood watery, brownish in colour.

Lungs:—Very pale; mediastinal gland swollen and congested.

Heart:—Normal.

Spleen:—Slightly swollen; splenic lymphatic glands swollen and deeply congested.

Liver:—Swollen slightly.

Kidneys:—One infarct size of a threepenny piece in left kidney.

Stomach:—Some erosions on mucous membranes.

Intestines:—Nil.

Bladder:—Distended with blood-coloured urine.

19. *Mule* 2214.—Four-year-old gelding.

Injected as above.

Result.—Reaction from the 18th day. Piroplasms present on the 15th, 16th and 18th days. Rings, rods and the lesions of poikilocytosis also noted.

20. *Mule* 2215.—Four-year-old gelding.

Result.—Reaction from the 13th to 34th days. Piroplasms noted on the 13th, 15th and 16th days. Rods, rings, points and the lesions of poikilocytosis also present.

21.—*Mule* 2217.—Three-year-old mare.

Injected as above.

Result.—Slight reaction from the 16th day. Piroplasms noted on the 13th, 16th, 22nd, 23rd and 26th days. Rings, rods, points and the lesions of poikilocytosis also present.

Argentine horse injected with blood of Transvaal horse foal—immune against piroplasma equi.

22. *Horse* 2095.—Four-year-old Argentine mare.

Injected on the 9th July, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of foal 1767.

Result.—Rise to 102 noted in the evening of the 11th day, and followed by a drop to sub-normal of 96.4. The temperature now recovered, recorded 106 in the evening of the 23rd day, and remained normal from the 31st day. Piroplasms noted on the 11th and 12th days, and again daily from the 14th to 17th days. From the 31st day to the 56th day piroplasma equi was frequently noted, occasionally accompanied with points and the lesions of poikilocytosis.

23. *Horse* 2096.—Three-year-old Argentine mare.

Injected on the 9th July, 1907, subcutaneously with 5 c.c. defibrinated fresh blood of foal 1767.

Result.—The 14th day after injection marked the commencement of a reaction, the temperature reached 102.6 two days later, but the animal died the following evening from rupture of spleen. Piroplasms noted on the 12th and 15th days. The examination of the animal about 12 hours previous to death showed the hindquarters to be slightly weak and the mucous membranes slightly yellow.

Post-mortem.—

Condition:—Good; uterus pregnant.
Lungs:—Normal.
Heart:—Normal.
Stomach:—Normal.
Kidneys:—Pale.
Liver.—Pale.
Spleen.—A rupture about four inches long on anterior surface.
Abdominal Cavity:—Full of blood.

Argentine mules injected with blood of Transvaal horse foal—immune against piroplasma equi.

Mules 2513 and 2516 were previously utilised for horse-sickness experiments on the 12th January, 1907, and injected on the 29th January, 1907, subcutaneously with 5 c.c. defibrinated fresh blood of Transvaal horse foal 1767.

24. *Mule* 2513.—Argentine gelding.

Injected as above.

Result.—No distinct reaction.

25.—*Mule* 2516.—Argentine mare.

Injected as above.

Result.—Sharp rise from the 4th day, and regaining normal three days later. Reaction from the 16th day. All microscopical examinations negative.

Transvaal donkey foal injected with blood of Transvaal donkey foal—immune against piroplasma equi.

26. *Donkey Foal* 2564.—Three months old; born on the station.

Injected on the 4th January, 1907, subcutaneously with 5 c.c. blood of donkey foal 2208.

Result.—No distinct reaction until the 23rd day. All examinations negative with the exception of the presence of a slight poikilocytosis on the 18th day.

Argentine horses injected with blood of Transvaal donkey foal—immune against piroplasma equi.

Note.—The following three horses, Nos. 2238, 2239 and 2244, were all injected subcutaneously on the 5th November, 1906, with 5 c.c. defibrinated fresh blood of donkey foal 2208.

27. *Horse* 2238.—Three-year-old Argentine gelding.

Injected as above.

Temperature.—Slight reaction from the 4th day, reaching 103 on the 9th day, and regaining normal on the 15th day,

Examinations.—Points noted on the 11th and 14th days, and 25th day piroplasms, accompanied with the lesions of a slight poikilocytosis, present on the 33rd day, the latter also being present the following day.

28. *Horse* 2239.—Seven-year-old Argentine gelding.

Injected as above.

Temperature.—No distinct reaction, the temperature only reaching the maximum of 102.2 on the 16th day.

Examinations.—One point noted on the 15th day, and two days later piroplasma equi was present. The lesions of a slight poikilocytosis were present on the 22nd day, and nine days later piroplasma equi again appeared.

29. *Horse* 2244.—Argentine gelding.

Injected as above.

Temperature.—No distinct reaction.

Examinations.—One point noted on the 10th day, and piroplasma equi present on the 13th day. A few points were again noted the following day and on the 24th day. Piroplasma equi again noticed on the 26th and 27th days.

Argentine horse injected with blood of Transvaal donkey—immune against piroplasma equi.

30. *Horse* 2243.—Four-year-old Argentine gelding.

Injected on the 16th October, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of donkey foal 2208.

Results.—Slight reaction from the 6th day, and second reaction from the 21st day, recording the maximum of 103.2 three days later. Points noted on 5th day; the animal was noted to be generally weak on the 7th day, with slightly yellow eyes, but an improvement was noted the following day. Points again noted on the 10th day, and piroplasms present in fair numbers the following day. Piroplasms now noted daily from the 14th to the 18th day, one rosette being present on the 16th day. Flagellated forms, points, forms, piroplasms, rosettes and the lesions of poikilocytosis frequently noted from the 19th to 33rd days. Piroplasms again seen on the 39th and 45th days. The lowest record of red corpuscles was 5,900,000 per c.m.m. on the 35th day.

Transvaal mule injected with blood of Transvaal donkey foal—immune against piroplasma equi.

31. *Mule* 2223.—Three-year-old mare. (Note.—Had previously been utilised in horse-sickness experiments.)

Injected on the 16th October, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of Transvaal donkey foal 2208.

Result.—Irregular reaction, recording, as the maximum temperature, 102.4 on the 21st day. Points noted on the 7th, 10th and 11th days. The lesions of poikilocytosis appeared on the 14th day, and from the 15th to the 18th days piroplasms were noted daily.

Argentine donkeys injected with blood of Transvaal donkey foal—immune against piroplasma equi.

Donkeys 2262 and 2263, both Argentine mares about two years old, had been utilised in horse-sickness experiments in September,

1906, and were injected on the 16th October, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of Transvaal donkey foal 2208.

32. *Donkey* 2262.—

Injected as above.

Result.—Irregular reaction. The maximum temperature of 104 obtained on the 7th day. Rings noted on the 10th day, and following by the appearance of piroplasms for two days. Piroplasms and poikilocytosis noted from the 14th to the 18th days.

33. *Donkey* 2263.—

Injected as above.

Result.—Irregular reaction. Piroplasms present on the 11th, 12th, 13th, 15th and 16th days. The lesions of poikilocytosis noted on the 15th, 19th and 26th days.

Results of animals inoculated with blood of Transvaal horse foals—immune against piroplasma equi (second generation).

3 Transvaal horse foals passed through the disease and recovered.
1 Transvaal donkey foal passed through the disease and recovered.
5 Transvaal mules passed through the disease, and one died of piroplasmosis.
6 Argentine horses passed through the disease, one being subsequently killed on account of pleuro-pneumonia, and one dying from rupture of spleen.
8 Argentine donkeys passed through the disease and recovered.
2 Argentine mules passed through the disease and recovered.

Results of animals inoculated with blood of a Transvaal donkey foal—immune against piroplasma equi (second generation).

1 Transvaal mule passed through a reaction and recovered.
1 Transvaal donkey foal passed through a reaction and recovered.
4 Argentine horses passed through a reaction and recovered.
2 Argentine donkeys passed through a reaction and recovered.

Experiment No. 12.

Fourth Generation.

Transvaal horse foal injected with blood of foal 1997*—immune against piroplasma equi.*

1. *Transvaal Horse Foal* 2619.—About four months old.

Injected subcutaneously on the 2nd February, 1907, with 5 c.c. defibrinated fresh blood of foal 1997.

Result.—Reaction from the 2nd day, reaching 106.6 on the 12th day, the evening temperature remaining high for the next four days. A second sharp rise was noted from the 19th day, the temperature recording 106.6 twenty-four hours later. Piroplasma equi noted on the 9th day. All other examinations negative.

Argentine mules injected with blood of foal 1997*—immune against piroplasma equi.*

Note.—The following mules (Nos. 2363, 2364, 2365, 2366 and 2367) were all injected subcutaneously on the 26th November, 1906, with 5 c.c. defibrinated fresh blood of foal 1997.

2. *Mule* 2363.—Four-year-old Argentine mare.

Injected as above.

Result.—Reaction from the 10th day. All microscopical examinations negative.

3. *Mule* 2364.—Five-year-old Argentine gelding.
Injected as above.
Result.—Reaction from the 10th day. The lesions of a slight poikilocytosis noted on the 16th day, and again on the 19th day, accompanied with points.
4. *Mule* 2365.—Four-year-old Argentine gelding.
Injected as above.
Result.—No distinct reaction. Poikilocytosis and points observed; piroplasma equi not present.
5. *Mule* 2366.—Three-and-a-half-year-old Argentine mare.
Injected as above.
Result.—Reaction from date of injection. Piroplasms noted on the 13th and 16th days, and poikilocytosis the 19th day.
6. *Mule* 2367.—Eighteen-month-old Argentine gelding.
Injected as above.
Result.—Very slight reaction from the 14th day. Piroplasms noted, but in very rare numbers, on the 16th day.

Note.—The following mules (Nos. 2508, 2509, 2511, 2512, 2534, 2535, 2536, 2538, 2540, 2541, 2542, 2543, 2544, 2545, 2546) were all injected subcutaneously on the 7th February, 1907, with 3 c.c. fresh defibrinated blood of foal 1997. These animals had previously been utilised for horse-sickness experiments.

7. *Mule* 2508.—Argentine mare.
Injected as above.
Result.—No distinct reaction.
8. *Mule* 2509.—Argentine mare.
Injected as above.
Result.—No distinct reaction.
9. *Mule* 2511.—Argentine mare.
Injected as above.
Result.—No distinct reaction.
10. *Mule* 2512.—Argentine gelding.
Injected as above.
Result.—Slight reaction from the 9th day.
11. *Mule* 2534.—Argentine mare.
Injected as above.
Result.—No distinct reaction.
12. *Mule* 2535.—Argentine gelding.
Injected as above.
Result.—No reaction.
13. *Mule* 2536.—Argentine mare.
Injected as above.
Result.—No distinct reaction.
14. *Mule* 2538.—Argentine mare.
Injected as above.
Result.—Slight reaction from the 10th day.
15. *Mule* 2540.—Argentine mare.
Injected as above.
Result.—No distinct reaction.
16. *Mule* 2541.—Argentine mare.
Injected as above.
Result.—Slight reaction from the 15th day.

17. *Mule* 2542.—Argentine mare.
Injected as above.
Result.—No reaction.

18. *Mule* 2543.—Argentine mare.
Injected as above.
Result.—No distinct reaction.

19. *Mule* 2544.—Argentine mare.
Injected as above.
Result.—No reaction.

20. *Mule* 2545.—Argentine mare.
Injected as above.
Result.—Slight reaction.

21. *Mule* 2546.—Argentine mare.
Injected as above.
Result.—Slight reaction.

Argentine donkeys injected with blood of foal 1997—*immune against piroplasma equi.*

Note.—The following donkeys, Nos. 2264, 2265, 2254, 2255, were all injected subcutaneously on the 26th November, 1906, with 5 c.c. defibrinated fresh blood of foal 1997.

22. *Donkey* 2264.—Three-year-old Argentine mare.
Injected as above.
Result.—A slight reaction. All microscopical examinations negative.

23. *Donkey* 2265.—Two-year-old Argentine mare.
Injected as above.
Result.—Slight reaction. All microscopical examinations negative.

24. *Donkey* 2254.—Three-year-old Argentine mare.
Injected as above.
Result.—Slight reaction.

25. *Donkey* 2255.—Three-year-old Argentine mare.
Injected as above.
Result.—Slight reaction from the 15th day. All microscopical examinations negative.

Argentine mules injected with blood of Transvaal foal 1998—*immune against piroplasma equi.*

The following Argentine mules, Nos. 2446, 2447, 2449, 2450, 2451, 2453, 2454, 2457, 2547, 2579, 2580, 2583, 2584, 2585, 2585, 2587, had all been utilised in horse-sickness experiments before being injected on the 7th February, 1907, subcutaneously with 3 c.c. defibrinated fresh blood of foal 1998.

26. *Mule* 2446.—Argentine gelding.
Injected as above.
Result.—Reaction from the 16th day. All microscopical examinations negative.

27.—*Mule* 2447.—Argentine mare.
Injected as above.
Result.—Reaction from the 16th day. All microscopical examinations negative.

28. *Mule* 2449.—Argentine mare.
Injected as above.
Result.—Reaction from the 17th day.

29. *Mule* 2450.—Argentine mare.
Injected as above.
Result.—Reaction from the 11th to 23rd days.

30. *Mule* 2451.—Argentine mare.
Injected as above.
Result.—Slight reaction from the 20th day.

31. *Mule* 2453.—Argentine gelding.
Injected as above.
Result.—Reaction from the 19th day. All microscopical examinations negative.

32. *Mule* 2454.—Argentine mare.
Injected as above.
Result.—Reaction from the 16th day. All microscopical examinations negative.

33. *Mule* 2457.—Argentine mare.
Injected as above.
Result.—Reaction from the 13th day.

34. *Mule* 2547.—Argentine mare.
Injected as above.
Result.—Slight reaction from the 15th day.

35. *Mule* 2579.—Two-and-a-half-year-old Argentine gelding.
Injected as above.
Result.—Temperature remained high from the date of injection, but was probably due to the previous injection of horse-sickness blood. Slight reaction from the 6th day, recording 104.6 twenty-four hours later.

36. *Mule* 2580.—Two-and-a-half-year-old Argentine mare.
Injected as above.
Result.—High temperature from date of injection, probably due to the previous inoculation of horse-sickness blood.

37. *Mule* 2583.—Argentine mare.
Injected as above.
Result.—Reaction from the 4th day.

38. *Mule* 2584.—Four-year-old Argentine mare.
Injected as above.
Result.—Reaction from the 7th day.

39. *Mule* 2585.—Two-and-a-half-year-old Argentine gelding.
Injected as above.
Result.—Slight reaction from the 6th day.

40. *Mule* 2586.—Four-year-old Argentine mule.
Injected as above.
Result.—No distinct reaction.

41. *Mule* 2587.—Argentine gelding about 18 months old.
Injected as above.
Result.—Slight reaction.

Transvaal donkey foal injected with blood of horse foal 1999—*immune against piroplasma equi.*

42. *Donkey Foal* 1773.—Nine-month-old stallion, and born on the station.

Injected on the 14th September, 1906, subcutaneously with 5 c.c. defibrinated fresh blood of foal 1999.

Result.—Irregular reaction. All microscopical examinations negative.

Argentine horses injected with blood of Transvaal horse foal 1999—*immune against piroplasma equi.*

Note.—Horses 2098 and 2102 were previously inoculated on the 9th July, 1906, with blood of foal 1991 and 1993 respectively (compare Experiment 1, Nos. 2 and 6), and were now tested on their immunity.

43. *Horse* 2098.—Three-year-old Argentine mare.

Injected subcutaneously on the 5th November, 1906, with 5 c.c. defibrinated fresh blood of foal 1999.

Result.—Slight reaction from the 14th day. One point noted on the 10th day, and again present on the 12th and 13th days. Piroplasma equi present for the first time on the 16th day, but in rare numbers, and again noted three days later, accompanied with the rosette form. All further examinations negative. Red corpuscles did not drop below 6,000,000 per c.m.m.

44. *Horse* 2102.—

Injected subcutaneously on the 5th November with 5 c.c. defibrinated fresh blood of foal 1999.

Result.—No distinct reaction, the temperature consistently remaining between 97 and 101.8. All blood examinations negative.

Transvaal mule injected with blood of Transvaal horse foal 1999—*immune against piroplasma equi.*

45. *Mule* 2212.—Three-year-old gelding.

Injected on the 5th November, 1906, subcutaneously with 5 c.c. blood of foal 1999.

Result.—Slight reaction. One point noticed on the 12th day, followed by the lesions of a slight poikilocytosis. Piroplasms only present on the 16th and 31st days, but in very rare numbers.

Argentine mule injected with blood of Transvaal horse foal 1999—*immune against piroplasma equi.*

The following mules, 2326, 2327, 2328, 2329, 2330, were all injected subcutaneously on the 5th November, 1906, with 5 c.c. blood of foal 1999.

46. *Mule* 2326.—Two-year-old Argentine gelding.

Injected as above.

Result.—On the 11th day the temperature rose from 100 in the morning to 104.8 in the evening, but fell almost immediately, and remained normal from the 18th day. Points noted on the 15th day, and the lesions of poikilocytosis appeared on the 27th day. One point also noted on the 31st day, but piroplasma equi were not present.

47. *Mule* 2327.—Three-and-a-half-year-old Argentine mare.
Injected as above.
Result.—Distinct reaction from the 9th day. Piroplasms noted on the 11th day, and together with points and the lesions of poikilocytosis, were fairly frequent during the next six days.

48. *Mule* 2328.—Two-year-old Argentine mare.
Injected as above.
Result.—Distinct reaction. Points noted on the 15th day, and the following day the lesions of a slight poikilocytosis present. One piroplasm, together with poikilocytosis, appeared on the 20th day, and this latter, together with points, were occasionally noted from the 26th to 31st days.

49. *Mule* 2329.—Eighteen-month-old Argentine mare.
Injected as above.
Result.—No distinct reaction. Piroplasms only noted on the 27th day, but points and the lesions of poikilocytosis were of fairly frequent occurrence.

50. *Mule* 2330.—Two-and-a-half-year-old Argentine mare.
Injected as above.
Result.—No distinct reaction. Piroplasms noted on the 25th and 27th days. The lesions of poikilocytosis also present.

The following Argentine mules, Nos. 2458, 2459, 2460, 2461, 2462, 2464, 2465, 2467, 2468, 2469, 2470, 2471, 2472, 2473, were all injected subcutaneously on the 7th February, 1907, with 3 c.c. blood of foal 1999, and had previously been utilised for horse-sickness experiments.

51. *Mule* 2458.—Argentine gelding.
Injected as above.
Result.—Sharp reaction immediately after injection, recording 105 in the evenings of the 2nd and 4th days. Remained normal from the 5th day.

52. *Mule* 2459.—Argentine mare.
Injected as above.
Result.—Reaction from the 13th day, reaching 105 in the evening of the 15th day. Second reaction from the 24th day.

53. *Mule* 2460.—Argentine gelding.
Injected as above.
Result.—Reaction from the 9th to 22nd days, recording as a maximum 103.4 in the 17th day.

54. *Mule* 2461.—Argentine mare.
Injected as above.
Result.—Reaction from the 18th day, reaching 105 in the same evening, and again the following 24 hours later. All microscopical examinations negative.

55. *Mule* 2462.—Argentine mare.
Injected as above.
Result.—Distinct reaction from the 15th day.

56.—*Mule* 2464.—Argentine gelding.
Injected as above.
Result.—No distinct reaction until the 17th day, when a rise to 102.3 was noted two days later.

57. *Mule* 2465.—Argentine mare.

Injected as above.

Result.—Reaction commenced on the 8th day, but was terminated two days later by the death of the animal from the sequel of piroplasmosis.

Post-mortem.—

Condition:—Fair; rigor mortis not set in; yellow infiltration of subcutaneous issue on shoulder.

Lungs:—Slightly œdematous.

Heart:—Brownish liquid in heartbag; white coagula of plasma; no lesions on endocard; gelatinous infiltration of sulci-transversalus.

Spleen:—Slightly enlarged and slightly congested.

Liver:—Thicker than normal, and congested.

Kidney.—Capsula of left kidney diffusely infiltrated with blood; left capsula not easily removed, and broke whilst removing; blood infiltrations in capsula only; kidney pale; right kidney capsula not easily slipped off; a white spot, the size of a walnut, and resembling an infarct, at beginning of cortex; malpighis bodies distinctly enlarged.

Stomach:—Contained a few superficial hæmorrhages.

Intestines.—Pale; cæcum and colon pale.

58. *Mule* 2467.—Argentine mare.

Injected as above.

Result.—Slight reaction from the date of injection, the animal dying on the 14th day from piroplasmosis.

Post-mortem.—

Condition:—Fair; rigor mortis not completely set in; watery blood of a brownish colour ran from shoulder after cutting; fascies and flesh somewhat of a brownish colour; serous membranes and all organs pale.

Lungs:—Clear yellow liquid in peritoneal cavity; lungs normal in appearance, but pale.

Heart:—Abnormal amount of liquid in heartbag; myocard soft and of a sepia colour; left endocard distended and hæmorrhagic; right endocard distended with a few hæmorrhages.

Spleen:—Considerably enlarged; pulpa soft; urinary bladder distended and contained red urine.

Liver:—Of a lightish brown colour.

Kidneys:—Capsula firmly fixed to subcutaneous tissue.

Stomach:—Filled with food; mucosa normal.

Intestines:—Cæcum and colon normal.

59. *Mule* 2468.—Argentine gelding.

Injected as above.

Result.—Slight reaction from the 17th day, recording 103 three days later, and remaining normal from the 24th day.

60. *Mule* 2469.—Argentine gelding.

Injected as above.

Result.—Slight reaction from the 9th day.

61. *Mule* 2470.—Argentine gelding.
Injected as above.
Result.—Reaction from the 5th to the 12th days.
62. *Mule* 2471.—Argentine gelding.
Injected as above.
Result.—Reaction from the 15th day, recording 104 on the 17th and 18th days.
63. *Mule* 2472.—Argentine gelding.
Injected as above.
Result.—Slight reaction from the 17th day.
64. *Mule* 2473.—Argentine mare.
Injected as above.
Result.—Reaction from the 5th day.

Transvaal donkey foal injected with blood of a Transvaal donkey foal—immune against piroplasma equi.

65. *Donkey Foal* 2550.—Three months old, and born on the station.
Injected on the 1st March, 1907, subcutaneously with 5 c.c. defibrinated fresh blood of foal 2564.
Result.—Reaction from the 7th day. Piroplasma equi noted on the 10th and 11th days, and two days later the lesions of poikilocytosis appeared.

Argentine horse injected with blood of Transvaal donkey foal—immune againt piroplasma equi.

66. *Horse* 2681.—Eight-year-old Argentine.
Injected on the 8th May, 1907, subcutaneously with 5 c.c. defibrinated fresh blood of foal 2564.
Result.—Slight reaction from the 11th to 19th days.

Results of animals inoculated with blood of Transvaal horse foals—immune against piroplasma equi (third generation).

1 Transvaal horse foal showed a reaction and recovered.
Of 55 Argentine mules the majority showed reactions, and two died, one from piroplasmosis and the other from the sequel of piroplasmosis.
4 Argentine donkeys showed reactions and recovered.
1 Transvaal donkey foal showed reactions and recovered.
1 Transvaal mule showed reactions and recovered.
Of two Argentine horses tested on their immunity, one showed a slight reaction; the other gave negative results.

Results of animals inoculated with blood of a Transvaal donkey foal—immune against piroplasma equi (third generation).

1 Transvaal donkey foal and 1 Argentine horse passed through a reaction and recovered.

Experiment No. 13.

Fifth Generation.

Argentine horse injected with blood of Transvaal donkey foal—immune against piroplasma equi.

1. *Horse* 2683.—Six-year-old Argentine mare.
Injected on the 8th May, 1907, subcutaneously with 5 c.c. of donkey foal 2550.
Result.—Slight reaction from the 7th day.

Transvaal donkey foal injected with blood of a Transvaal donkey foal—immune against piroplasma equi.

2. *Donkey Foal* 2551.—Three months old, and born on the station.

Injected on the 26th March, 1907, subcutaneously with 5 c.c. defibrinated fresh blood of Transvaal donkey foal 2550.

Temperature.—Very slight reaction.

Examinations.—The lesions of poikilocytosis noted on the 21st day.

3. *Donkey Foal* 2494.—Four-month-old foal, and born on the station.

Injected on the 25th April, 1907, subcutaneously with 5 c.c. defibrinated fresh blood of foal 2550.

Temperature.—Reaction from the 13th day.

Examinations.—Piroplasma equi noted on the 13th day, followed by the lesions of poikilocytosis on the 14th day.

Argentine mules injected with blood of a Transvaal donkey foal—immune against piroplasma equi.

4. *Mule* 2539.—Argentine gelding. (Note.—Mules 2539 and 2597 had previously been utilised for horse-sickness experiments.)

Injected on the 26th March subcutaneously with 5 c.c. defibrinated fresh blood of foal 2550.

Temperature.—Short reaction from the 9th to 13th days, recording as a maximum 103.8 on the 11th day after injection.

Examinations.—Piroplasma equi only noted on the day of the maximum temperature.

5. *Mule* 2597.—Two-and-a-half-year-old Argentine mare.

Injected on the 26th March subcutaneously with 5 c.c. defibrinated fresh blood of foal 2550.

Temperature.—Slight reaction. Piroplasma equi noted on the 15th day, and the lesions of poikilocytosis appeared the next day.

Argentine donkey injected with blood of a Transvaal donkey foal—immune against piroplasma equi.

Note.—The following donkeys, Nos. 2432, 2433, 2435, 2437, 2439, 2441, 2444, 2445, were all injected subcutaneously on the 26th March, 1907, with 5 c.c. defibrinated fresh blood of foal 2550.

6. *Donkey* 2432.—Five-year-old Argentine mare.

Injected as above.

Temperature.—Reaction from the 7th day, recording 104 two days later.

Examinations.—All negative.

7. *Donkey* 2433.—Four-year-old Argentine gelding.

Injected as above.

Temperature.—Slight reaction.

Examinations.—All negative.

8. *Donkey* 2435.—Four-and-a-half-year-old Argentine gelding.

Injected as above.

Temperature.—Sharp rise noted from the 13th day, recording 104.2 on the evening of the 14th day, and regaining normal two days later.

9. *Donkey* 2437.—Two-and-a-half-year-old Argentine mare.
Injected as above.
Temperature.—Reaction from the 6th day, reaching 105 three days later, and 104 in the evening of the 13th day.
Examinations.—Piroplasms noted on the 9th, 10th and 14th days.
10. *Donkey* 2439.—Two-and-a-half-year-old Argentine gelding.
Injected as above.
Temperature.—No distinct reaction.
Examinations.—All negative.
11. *Donkey* 2441.—Three-year-old Argentine mare.
Injected as above.
Temperature.—Short reaction from the 14th day, lasting for three days.
Examinations.—All negative.
12. *Donkey* 2444.—Three-and-a-half-year-old Argentine gelding.
Injected as above.
Temperature.—No distinct reaction.
Examinations.—All negative.
13. *Donkey* 2445.—Six-year-old Argentine gelding.
Injected as above.
Temperature.—No reaction.
Examinations.—All negative.

Transvaal horse foal injected with blood of a Transvaal horse foal—immune against piroplasma equi.

14. *Foal* 2620.—Filly obtained from S.A.C.
Injected on the 30th May, 1907, subcutaneously with 5 c.c. defibrinated fresh blood of foal 2681.
Temperature.—Very slight reaction from the 8th day.
Examinations.—The lesions of poikilocytosis noted on the 12th day.

Results of animals injected with blood of a Transvaal donkey foal—immune against piroplasma equi (fourth generation).

1 Argentine horse showed a reaction and recovered.
2 Transvaal donkey foals showed a reaction and recovered.
2 Argentine mules showed a reaction and recovered.
Of 8 Argentine donkeys, the majority showed a reaction, and all recovered.
1 Transvaal horse foal, injected with blood of an immune Transvaal horse foal (fourth generation), passed through a piroplasmosis reaction and recovered.

Experiment No. 14.

Sixth Generation.

Transvaal horse foal injected with blood of a Transvaal horse foal—immune against piroplasma equi.

1. *Horse Foal* 2408.—About six months old.
Injected on the 30th May, 1907, subcutaneously with 5 c.c. defibrinated fresh blood of horse foal 2683.
Result.—Slight reaction from the 5th to 21st day. Piroplasma noted on the 6th, 7th and 11th days. The lesions of poikilocytosis present on the 8th, 9th and 13th days.

Transvaal horse foal injected with blood of Transvaal donkey foal—immune against piroplasma equi.

2. *Foal* 2707.—Three-month-old colt, and obtained from S.A.C.

Injected on the 30th May subcutaneously with 5 c.c. defibrinated fresh blood of foal 2551.

Temperature.—Sharp rise from the 3rd day to 105 in the evenings of the 5th and 6th days, followed by a fall to 100 two days later, and a rise to 104.6 on the 10th day.

Examinations.—The lesions of poikilocytosis noted on the 5th, 7th, 9th and 11th days; piroplasma equi in rare numbers noted on the 8th day.

Transvaal horse foal injected with blood of Transvaal donkey foal—immune against piroplasma equi.

3. *Foal* 2767.—Six-month-old colt, and obtained from the S.A.C.

Injected on the 30th May, 1907, subcutaneously with 5 c.c. defibrinated fresh blood of donkey foal 2494.

Temperature.—Sharp reaction from the 8th day, reaching 104 in the evening two days later. The 21st day marked the commencement of a second reaction, but the animal died four days later of horse-sickness.

Examinations.—The lesions of poikilocytosis noted on the 9th, 11th and 14th days.

Result of two Transvaal horse foals injected with blood of Transvaal donkey foals (fifth generation), one died of horse-sickness.

One Transvaal horse foal injected with blood of a Transvaal horse foal (fifth generation), immune against piroplasma equi, passed through a reaction and recovered.

Summary of Results.

(a) *Origin Horse Blood.*
Injections with blood of horses and horse foals.

Of 4 Transvaal horse foals, injected with immune blood (origin), none died.

Of 8 Argentine horse mares, injected with blood of 1st generation,
- 1 died from gangrenous pneumonia (probably ship's pneumonia). (This mare was heavy in foal.)
- 1 died from gastro-enteritis (probably horse-sickness.)
- 2 died from piroplasmosis. These mares were in foal, and this no doubt had some connection with the mortality.

(b) *Origin Mule Blood.*
Injection with blood of horse foals.

Of 9 Transvaal horse foals, injected with blood of 1st to 7th generations, none died.

Of 2 Argentine horses, injected with blood of 1st generation,
- 1 died of debility, complicated with piroplasmosis, and
- 1 died of syncope.

Of 2 Argentine horses, injected with blood of 2nd generation,
- 1 died of pneumonia (probably ship's pneumonia).

1 Argentine horse, injected with blood of 6th generation, died from debility, complicated with piroplasmosis.

Of 12 Argentine donkeys, injected with blood of 1st and 2nd generations, 1 died of debility. This mare slipped her foal during the reaction.
Of 5 Transvaal mules, injected with blood of 2nd generation, none died.
Of 67 Argentine mules, injected with blood of 2nd, 3rd and 4th generations, 1 died of pneumonia (probably ship's pneumonia).

(c) *Origin Donkey Blood.*

1.—*Injections with donkey blood.*

1 Transvaal horse foal, injected with donkey blood (origin), recovered.
7 Argentine horses, 3 Argentine mules and 3 Argentine donkeys, tested on their immunity by injection of donkey foal blood (origin), recovered.

2.—*Injections with horse blood.*

2 Transvaal horse foals, injected with blood of a Transvaal horse (1st generation), recovered.

3.—*Injections with horse foal blood.*

1 Transvaal horse, injected with blood of Transvaal horse foal (1st generation), recovered.
7 Transvaal horse foals, injected with blood of Transvaal horse foal (2nd to 6th generations), recovered.
Of 6 Transvaal mules, injected with blood of Transvaal horse foals (2nd and 3rd generations), 1 died of piroplasmosis.
3 Transvaal donkey foals, injected with blood of Transvaal horse foals (1st, 2nd and 3rd generations), recovered.
Of 8 Argentine horses, injected with blood of a Transvaal horse foal (1st and 2nd generations), 2 died from rupture of the spleen, and 1 was killed on account of pleuro-pneumonia.
2 Argentine horses were tested on their immunity by injection of blood of Transvaal horse foal (3rd generation), and recovered.
Of 57 Argentine mules, injected with blood of Transvaal horse foal (1st, 2nd and 3rd generations), 1 died from piroplasmosis and 1 from sequel of piroplasmosis.
12 Argentine donkeys, injected with blood of Transvaal horse foal (2nd and 3rd generations), recovered.

4.—*Injections with donkey foal blood.*

Of 2 Transvaal horse foals, injected with donkey foal blood (2nd and 5th generations), 1 died of horse-sickness, contracted spontaneously.
1 Transvaal mule, injected with blood of Transvaal donkey foal (2nd generation), recovered.
2 Transvaal donkey foals, injected with blood of a Transvaal donkey foal (2nd and 3rd generations), recovered.
6 Argentine horses, injected with blood of Transvaal donkey foals (2nd, 3rd and 4th generations), recovered.
2 Argentine mules, injected with blood of Transvaal donkey foals (4th generation), recovered.
Of 10 Argentine donkeys, injected with blood of Transvaal donkey foals (2nd and 4th generations), none died.

From this summary several points are noticeable, namely :—

(*a*) 3 Argentine horses and 1 Argentine mule died from pneumonia, probably caused by an infection of "ship's pneumonia," one mare also being heavy in foal,

(*b*) 2 Argentine mare horses, heavy in foal, died from piroplasmosis, and undoubtedly the pregnancy, together with the reaction, must be held responsible.

(*c*) 2 Argentine horses died from piroplasmosis, complicated with debility.

(*d*) 2 Argentine horses died from rupture of the spleen.

These points suggest certain precautionary measures which should be taken previous to inoculation :—(*a*) Animals imported from oversea should not be inoculated until all danger of an infection with ship's pneumonia has been removed ; (*b*) mares heavy in foal should not be inoculated ; (*c*) animals in poor condition should not be inoculated ; (*d*) the contingency must always be expected that Argentine horses and mules may die of rupture of spleen, as they are very wild, and stabling often causes them to contract serious injuries.

Deaths wholly or partially caused by the piroplasmosis reaction.

(a) *Origin Horse Blood.*

3 Argentine horses (injected with horse foal blood of 1st generation).

(b) *Origin Mule Blood.*

1 Argentine horse (injected with horse foal blood of 1st generation).
1 Argentine horse (injected with horse foal blood of 6th generation).

(c) *Origin Donkey Blood.*

1 Transvaal mule (injected with horse foal blood of 2nd generation).
2 Argentine mules (injected with horse foal blood of 3rd generation).

In no instance did the injection of donkey foal blood cause the death of an animal.

Note.—All animals which survived the vaccination were exposed to natural infection soon after the conclusion of the reaction. These animals have been kept under close daily observation, and no deaths or relapses have been reported.

Conclusions.

(1) The inoculation of animals with horse foal blood of 1st, 2nd and 3rd generations caused a mortality of 7 out of 186, or 4 per cent.

(2) The inoculation of animals with horse foal blood of 4th, 5th, 6th, 7th and 8th generations caused a mortality of 1 in 16, or 6 per cent.

(3) The inoculation of animals with donkey foal blood of 2nd to 6th generation caused no mortality amongst 25 animals.

(4) The reactions caused by injection of horse foal blood were more severe than those given by injection of donkey foal blood.

(5) No cases of relapses after discharge have occurred, proving that the immunity given by the injection of donkey foal blood is as good as that afforded by horse foal blood.

(6) For further immunisation purposes, therefore, I recommend the passing of blood originating from a natural infection of a donkey with piroplasma equi through donkey foals, and to use 1 c.c. blood of that obtained from the 4th generation and upwards, bearing in mind the precautionary measures mentioned above.

(7) Finally, all foals kept for tapping purposes, and used in connection with the inoculation, must be kept free from ticks. This precaution has been carried out at this Laboratory with all foals used in the experiments, and it stands to reason that a reinfection by means of ticks would increase the virulency of the blood which is to be used as vaccine.

INOCULATION OF SHEEP AGAINST BLUE TONGUE AND THE RESULTS IN PRACTICE.

The possibility of obtaining a virus and serum which would immunise sheep against blue tongue was mentioned by Spreull some few years ago, and in the article entitled "Blue Tongue in Sheep," included in my Annual Report for 1904-5, details were given of several experiments which were conducted in the way he indicated.

However, the reaction necessary to produce an active immunity was not noticed in these injected sheep, and as it was probable that the large dose of serum given was responsible, in the next experiments I reduced the quantity.

The virus was now passed through several generations of sheep, the details of which are given hereunder :—

Generation of Virus.	No. of Sheep Injected.		Deaths During Injection.	
1	9		1	
2	7		2	
3	4		1	
4	3		1	
5	3		1	
6	3		0	
7	12		0	
8	11		1	
9	35		2	
10	6		1	
	——	93	——	10
11	49		0	
12	19		0	
13	28		0	
14	13		0	
15	24		0	
16	17		0	
17	31		0	
18	118		0	
	——	299	——	0

From this table it will be seen that after the 10th generation the injection of virus did not cause any deaths. In addition to this, the temperature charts proved that all animals passed through a typical, yet rather severe, form of blue tongue, but no clinical symptoms were observed.

The percentage of mortality from the first 10 generations, out of 93 inoculated, was 11 per cent., and from the 11th to the 18th generation of 299 inoculated, *nil*.

After testing the sheep from the 11th generation onwards, and finding that the immunity given was equal to that obtained from a natural attack, I decided to introduce this attenuated virus into practice in the form of a vaccine. This was done in February, 1907, and at the end of the season statistics were collected as to the results obtained.

Unfortunately only a small percentage of results came to hand, which are given hereunder. The deaths following vaccination have been divided into those occurring—(1) within 9 days from inoculation; (2) from 10th to 14th days; and (3) after 14 days from inoculation.

The reason for this being that the whole course of blue tongue averages 14 days, and this period must, therefore, be allowed for the reaction consequent on the vaccine before immunity is finally established. An animal already suffering from the disease at the date of vaccination would probably die within 9 days.

Deaths occurring between the 10th and 14th days are not considered as a result of natural infection, and the vaccine is probably held responsible. At the same time I must point out that of the 299 sheep vaccinated at this station, none died, but when the vaccine is used in practice on thousands of animals, some deaths are certain to occur. Mortality after the reaction has finished (that is to say, from the 14th day onwards) have been considered as relapses (breakdowns in immunity).

As in many instances farmers did not inoculate their whole flocks, statistics were also collected regarding the mortality amongst the non-vaccinated animals, and, for the purposes of comparison, have been embodied in the following return :—

RETURNS OF MORTALITY AMONGST VACCINATED SHEEP AS COMPARED WITH MORTALITY OF NON-VACCINATED SHEEP RUNNING ON THE SAME FARM.

DISTRICT.	VACCINATED SHEEP.				NON-VACCINATED SHEEP.		PER CENT. OF DEATHS AMONGST	
	No. Vaccinated.	No. which Died within.		No. which Died after 14 Days.	Number.	No. which Died.	Vaccinated Animals.	Non-Vaccinated.
		1—9 Days.	10—14 Days.					
							Per cent.	Per cent.
Ermelo ...	1,906	12	17	2	3,228	336	0·9	10·
Heidelberg ...	23	1	0	2	3,542	283	0	8·
Middelburg ...	142	39	6	0	2,204	599	3·0	27·
Lydenburg ...	966	0	1	3	2,289	200	0·1	9·
Marico ...	36	1	0	0	400	16	0	4·
Waterberg ...	1,200	0	0	0	Not	stated.	0	
Rustenburg ...	11	0	0	0	,,	,,	0	
Pretoria ...	1,065	19	1	0	,,	,,	0·1	—
Barberton ...	10	0	0	0	130	Nil	0	0
Standerton ...	106	2	1	0	4.425	383	0·9	9·
Volksrust ...	10	0	0	0	—	—	0	—
	5,875	74	26	7	16,218	1,817	0·4	11·0

Naturally these figures only represent a small minority of the sheep in the Transvaal, and the number vaccinated during the season, but the return is accurate as regards the statistics at my disposal, and the results may safely be considered as typical for the Colony. The percentage of deaths due to the vaccination is 0.4 per cent., and relapses amount to 0.1 per cent.

These results show very clearly the advantage vaccinated sheep have over susceptible animals.

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